An Exploratory Study of Endophenotype Markers for Schizophrenia

Picchioni, Mark

Awarding institution:
King's College London

Title: An Exploratory Study of Endophenotype Markers for Schizophrenia

Author: Mark Picchioni

An Exploratory Study of Endophenotype Markers for Schizophrenia

Mark Michael Picchioni

Submitted in partial fulfillment of the degree of
Doctorate of Philosophy PhD
King's College London
Institute of Psychiatry

Abstract:

Genetic studies of schizophrenia have often been frustrated by the aetiological and pathological complexity of the disorder. One research strategy to address this issue is the use of endophenotype markers.

The aim of this PhD thesis is to explore the validity of selected candidate endophenotype markers for schizophrenia by establishing the evidence that they may have some or indeed any relationship with the genetic risk for the disorder.

I have studied a unique twin and family population, to describe the distribution of a variety of deficits as candidate markers in patients, their unaffected co-twins and relatives, and healthy controls, to explore the evidence for genetic and environmental influences on their distribution.

I selected neurological soft signs, personality and social development, grey and white matter cerebral volumes, and regional neural activity during a phonological verbal fluency task.

The results were most compelling for the developmental and verbal fluency markers, supporting their role as endophenotype markers. In comparative terms, this may relate more to methodological factors, than the markers inherent properties. All of the markers demonstrated that they met many endophenotype criteria.

For Lily, Ines, Xanthe and Sarah

Acknowledgements:

A PhD is a journey, and I have had the fantastic pleasure to travel with some amazing, bright, engaging, energetic, supportive and encouraging people on this particular trip. I would in particular like to say huge thanks to those who I worked with most closely in the Twin Study, Timothea Toulopoulou, Mei Hua-Hall, Xavier Chitnis, Neeltje van Haren, Sheena Waters-Metenier, Sheena Owens, Andreina Pauli, Nadia Davies and Tracey Ribchester, Bernard Freeman and David Collier. Thanks also to Colm McDonald, Muriel Walshe, Elvira Bramon Madiha Shaikh, and Chris Chaddock in the Family Study. Neuroimaging was such a large part of this project and it is so easy to be complacent about all the support and organisation that magically happened down in Mapother House to make scanning the twins so successful, special thanks to Dave Gasston, Chris Andrew, Steve Williams, Mick Brammer, Vincent Giampietro, Gareth Barker, Andy Simmons, and all the radiographers at what was Mapother House, and now the CNS, thanks for helping me look after the twins.

Working on a PhD demands all sorts of organisational skills that I never had, so tremendous thanks to Sandra Whitehead and Averil Baxter, who were always there to support and help, when I didn't know what form to fill in, who should sign it, or where to send it.

When it came to my pastoral care, who could have asked for a better bunch than my friends at the IoP, especially Paul Allen, Marc Seal, Isabel Valli, Stefan Borgwardt, Richard Kanaan, Ulrich Ettinger, Francesco Carletti, Paolo Fusar-Poli, Lucia Valmaggia, and of course from back in the day, Sukhi Shergill, Cindy Fu, Katsunori Matsumoto and Edson Amaro Jnr.

Finally to my supervisors Prof Philip McGuire and Prof Sir Robin Murray, for frankly making every day at the Institute a pleasure, interesting, and I don't think ever saying no. Thank you for having the ideas, imagination and determination that made it all happen.

I would like to acknowledge the financial support of the Wellcome Trust, who stumped up the cash for three and half years, through a Research Training Fellowship (064971) and the Trustees of Brain and to Pfizer for travel and conference support.

Table of Contents

List of Figures

Reprinted from: Picchioni MM, Toulopoulou T, Landau S, Davies N, Ribchester N, Murray R.M. (2005)Neurological abnormalities in twins. Biological Psychiatry 59, 4 341-348 with permission from Elsevier..

Figure 3.3.
Box plots demonstrating distribution of Integration NA score within groups.
Disc, Discordant; N, Number of subjects; Min, Minimum; Max, Maximum; NA, Neurological Abnormality.
Reprinted from: Picchioni MM, Toulopoulou T, Landau S, Davies N, Ribchester N, Murray R.M. (2005)Neurological abnormalities in twins. Biological Psychiatry 59, 4 341-348 with permission from Elsevier.

Figure 5.1
Plot of Whole Brain Volume St George's against The Maudsley Sites

Figure 5.2
Plot of Grey, White and CSF Volume St George's against The Maudsley Sites

Figure 5.3
Bland-Altman Plots of Whole Brain, Grey & White Matter Volumes St George's against The Maudsley Sites

Figure 5.4
Mean Adjusted Whole Brain Volume by Group

Figure 5.5
Mean Adjusted Grey Matter Brain Volume by Group

Figure 5.6
Mean Adjusted White Matter Brain Volume by Group

Figure 6.1
Main Effect of Group - Across 6 Groups: MZ Concordant Schizophrenia ≥ MZ Discordant Schizophrenia ≥ DZ Twin & Sibling Discordant Schizophrenia ≥ MZ Discordant Non-psychotic ≥ DZ Twin & Sibling Discordant Non-psychotic ≥ Healthy Controls. Coronal, Sagittal, Axial Views, illustrating the left inferior frontal gyrus cluster.

Figure 6.2
Extracted Signal Intensity (Mean Cluster SSQ Response) across the 6 Groups at the Left Inferior Frontal Gyrus. Coordinates: Tal(x)=-43, Tal(y)=15, Tal(z)=-2.
Abbreviations: MZ, monozygotic, DZ, dizygotic, Cc, concordant, Dc, discordant, Scz, schizophrenia.

Figure 6.3
Extracted Signal Intensity (Mean Cluster SSQ Response) across the 6 Groups at the Left Hippocampal/Parahippocampal Gyrus. Coordinates: Tal(x)=-29, Tal(y)=-52, Tal(z)=-2.
Abbreviations: MZ, monozygotic, DZ, dizygotic, Cc, concordant, Dc, discordant, Scz, schizophrenia.

Figure 6.4
Extracted Signal Intensity (Mean Cluster SSQ Response) across the unaffected Subjects in the Left Superior Temporal Gyrus. Coordinates: Tal(x)=-58, Tal(y)=-11, Tal(z)=4.
Abbreviations: MZ, monozygotic, DZ, dizygotic, Dc, discordant.

Figure 6.5
Extracted Signal Intensity (Mean Cluster SSQ Response) across the unaffected Subjects in the Right Middle Temporal Gyrus. Coordinates: Tal(x)=29, Tal(y)=-52, Tal(z)=15.
Abbreviations: MZ, monozygotic, DZ, dizygotic, Dc, discordant.

Fig 7.1
This shows in simplified, schematic terms some of the possible relationships between putative endophenotypes, gene and disease. In reality, different combinations of these simplified scenarios are likely. G, genes; Enviro, environmental factors; Endo, putative endophenotype; P, disease phenotype.
Reprinted by permission from Macmillan Pulishers Ltd: Molecular Psychiatry. JTR Walters and MJ Craddock. Endophenotypes in psychiatric genetics (2007). Molecular Psychiatry 12; 886-890.

Figure 7.2
(a) A liability-index model for endophenotypes (EPs). Genetic variance VG influences both the EP and psychiatric disorder (PD). These observed variables also have residual variation, RVEP and RVPD, due to other sources.
(b) A mediational model for EPs. Genetic variance causes variation in the EP, which in turn causes variation in PD. EP and PD have residual variance components RVEP and RVPD, respectively.
Reprinted by permission from Macmillan Publishers Ltd: Molecular Psychiatry. KS Kendler and MC Neale. Endophenotype: a conceptual analysis (2010). Molecular Psychiatry 15; 789-797.

Figure 7.3
A nonexclusive mediational model for endophenotypes (EPs) including a direct causal path from genes to the psychiatric disorder (aPD) that does not pass through EP.
Reprinted by permission from Macmillan Pulishers Ltd: Molecular Psychiatry. KS Kendler and MC Neale. Endophenotype: a conceptual analysis (2010). Molecular Psychiatry 15; 789-797.

List of Tables

Legend Table 4.4
Abbreviations: MZ, monozygotic; DZ, dizygotic ; CSA, childhood social adjustment ; ASA, adolescent social adjustment ; PSST Premorbid Schizotypal traits.
Note: The schizophrenia cross-twin correlation (SZtw1–SZtw2) is constrained to be .92 in MZ twins and .515 in DZ twins based on the point estimates of meta-analysis results, and the thresholds on the liabilities are fixed to a prevalence of 1%. Intervals including 0 indicate non-significance.

Table 4.5
Additive genetic, dominant genetic, shared and specific environmental estimates (with 95% CI) of the AD/CE genetic model for CSA, ASA, and PSST

Legend Table 4.5
Abbreviations: CSA, childhood social adjustment ; ASA, adolescent social adjustment ; PSST Premorbid Schizotypal traits.
Note: a2, d2, c2 and e2: broad heritability, dominant genetic, shared and non-shared environmental. Confidence intervals including zero indicate non-significance.
Parameters for schizophrenia are fixed based on a prevalence of 1% and the following genetic model: h2=.81, c2=.11, e2=.08

Table 4.6
The phenotypic correlations between schizophrenia and the developmental and personality scores (rph), the decomposed sources of the correlations (rph-a, rph-c, rph-e) predicted by the AC/DE models and correlation estimates (with 95% CI)

Legend 4.6
Abbreviations: rph: total phenotypic correlation; rph-a, rph-c, rph-e, phenotypic correlation due to additive genetic, shared and unique environmental influences. ra, re, re, correlation between additive genetic, shared and specific environmental factors. Confidence intervals including zero indicate non-significance. Fixed genetic model for Schizophrenia used: h2=.81, c2=.11, e2=.08

Table 5.1
Demographics of Experimental Groups

Legend Table 5.1
Note: Data reflect mean (and standard deviation) unless otherwise stated.

Table 5.2
Clinical Ratings of Experimental Groups

Legend Table 5.2
Note: Data reflect mean (and standard deviation) unless otherwise stated. SAPS=Scale for the Assessment of Positive Symptoms; SANS=Scale for the Assessment of Negative Symptoms; CPZ=chlorpromazine; n/a = not available.

Table 5.3
Intra Class Correlations for Whole Brain, Grey and White Matter Volume

Table 5.4
Adjusted Brain Volumes by Group

Legend Table 5.4

Note: Data reflect mean (and standard deviation) of adjusted brain volumes in ml by Group. Whole Brain = whole brain volume; Grey = grey matter volume; White = white matter volume.

Table 5.5
Between Group Tests

Legend Table 5.5
MZ Conc = MZ Concordant; MZ Disc Ill = MZ Discordant Ill; MZ Disc Well= MZ Discordant well. Whole Brain = whole brain volume; Grey = grey matter volume; White = white matter volume. *p<0.05, ** p<0.017, *** p<0.0125 corrected for multiple comparisons.

Table 5.6
Intraclass Correlations (ICC) by Twin Category

Legend Table 5.6
Intraclass correlations (ICC) coefficients (and 95% confidence interval) by twin category. Whole Brain = whole brain volume; Grey = grey matter volume; White = white matter volume.

Table 6.1
Demographics of Twins & Siblings

Legend Table 6.1
Abbreviations: Cc, concordant; Dc, discordant; MZ, monozygotic; DZ, dizygotic ; Scz schizophrenia

Table 6.2
Main Effect Across 6 Groups: Verbal Fluency vs. Baseline

Legend Table 6.2
Abbreviations: MZ, monozygotic, DZ, dizygotic, Cc, concordant, Dc, discordant, Scz, schizophrenia, L, left, R, right. All clusters reported at voxel p=0.05 and cluster p=0.05 for the former and p=0.02 for latter contrast, yielding less than one false positive cluster respectively. Only the cluster with the largest number of voxels in each region is reported, and is limited to clusters of more than five voxels. Talairach coordinates refer to the voxel with the largest sum of squares ratio, a measure of power of neural response, in each cluster.

Table 6.3:
Effect Across Unaffected Subjects: Verbal Fluency vs. Baseline

Legend Table 6.3
Abbreviations: MZ, monozygotic, DZ, dizygotic, Cc, concordant, Dc, discordant, Scz, schizophrenia, L, left, R, right. All clusters reported at voxel p=0.05 and cluster p=0.02, yielding less than one false positive cluster. Only the cluster with the largest number of voxels in each region is reported, and is limited to clusters with more than five voxels. Talairach coordinates refer to the voxel with the largest sum of squares ratio, a measure of power of neural response, in each cluster.

Table 6.4
MZ Discordant Schizophrenia > MZ Discordant Non-psychotic. Verbal Fluency vs. Baseline

Legend Table 6.4
Abbreviations: MZ, monozygotic, DZ, dizygotic, L, left, R right. All clusters reported at voxel p=0.05 and cluster p=0.01, yielding less than one false positive cluster. Only the cluster

with the largest number of voxels in each region is reported, and is limited to clusters with more than five voxels. Talairach coordinates refer to the voxel with the largest sum of squares ratio, a measure of power of neural response, in each cluster.

Table 6.5
Cross-twin/sibling Within Trait and Cross-twin/sibling Cross-trait Correlations (r & 95% CI)

Legend Table 6.5
Abbreviations: MZ, monozygotic; DZ, dizygotic
Note: The schizophrenia cross-twin correlation (SZtw1–SZtw2) is constrained to be .92 in MZ twins and .515 in DZ twins/siblings based on the point estimates of meta-analysis results, and the thresholds on the liabilities are fixed to a prevalence of 1%.
Intervals including 0 indicate non-significance.

Table 6.6.
Additive genetic, common and specific environmental estimates (with 95% CI) of full ACE genetic model

Legend Table 6.6
Abbreviations: MZ, monozygotic; DZ, dizygotic
Note: h2,c2, and e2: heritability, shared and non-shared environmental. Confidence intervals including zero indicate non-significance.
Parameters for schizophrenia are fixed based on a prevalence of 1% and the following genetic model: h2=.81, c2=.11, e2=.08

Table 6.7
The phenotypic correlations between schizophrenia and regional brain activity (r_{ph}), the decomposed sources of the correlations ($r_{ph\text{-}a}$, $r_{ph\text{-}c}$, $r_{ph\text{-}e}$) predicted by the ACE models.

Legend Table 6.7
Note: $\boldsymbol{r_{ph:}}$ total phenotypic correlation; $\boldsymbol{r_{ph\text{-}a}}$, $\boldsymbol{r_{ph\text{-}c}}$, $\boldsymbol{r_{ph\text{-}e}}$, phenotypic correlation due to additive genetic, shared environmental and specific environmental influence.
Confidence intervals including zero indicate non-significance.
Fixed genetic model for Schizophrenia used: h^2=.81, c^2=.11, e^2=.08

Papers Arising from this Thesis

Chapter 1
Picchioni M, Murray R. Clinical Review: Schizophrenia. (2007) British Medical Journal; 335:91-95.

Chapter 3
Picchioni MM, Toulopoulou T, Landau S, Davies N, Ribchester N, Murray R.M. (2005)Neurological abnormalities in twins. Biological Psychiatry 59, 4 341-34869

Chapter 4
M.M. Picchioni, M. Walshe, T Toulopoulou, C. McDonald, M Taylor, S Waters-Metenier, E. Bramon, A Regojo, RM Murray, F Rijsdijk. (2010) Genetic modelling of childhood social development and personality in twins and siblings with schizophrenia Psychological Medicine, 40; 8: 1305-1316. 2009 © Cambridge Journals, reproduced with permission.

Chapter 1

1. An Introduction to Schizophrenia and Endophenotypes

1.1. Abstract

1.1.1. In this introductory chapter I will describe schizophrenia and introduce the concept of endophenotypes.

1.2. What is schizophrenia?

1.2.1. 'Schizophrenia' is a psychiatric disorder and falls within the scope of psychotic illnesses. This in simple terms means that the patient at some point develops symptoms that lead them to misinterpret reality. More specifically schizophrenia is characterised by the presence of certain symptoms and signs, in particular delusions and hallucinations, and thought disorganisation.

1.2.2. The name schizophrenia was derived from the early observation that the illness is typified by '*the disconnection or splitting of the psychic functions*' (Bleuler 1911). Unfortunately, this has lead to the widespread common misconception that the illness is somehow characterised by a split personality.

1.3. How Has the Concept of Schizophrenia Evolved?

1.3.1. In the early 1800s many believed in a single psychotic illness '*Einheitpsychose*', though by the early 1850s two French psychiatrists Falret and Morel had distinguished '*folie circulaire'* what we now think of as manic depressive psychosis from '*demence precoce' (Morel 1860; Falret 1878),* a progressive disorder, characterised by social withdrawl, self neglect and bizarre behaviour. In Germany Hecker later employed the term '*hebephrenia*' to describe a very similar clinical concept to '*demence precoce'(Hecker 1871),* while his mentor Kahlbaum proposed '*catatonia*' to describe a presentation of psychosis but dominated by episodes of abnormal movement and posture (Kahlbaum 1863).

1.3.2. Emil Kraepelin subsequently united the concepts of '*hebephrenia'* and '*catatonia'* with '*paranoia'* into a single disorder '*dementia praecox'* (Kraepelin 1913). Kraepelin too believed that this was a progressive disorder beginning in early adult life, and characterised by delusions and hallucinations, disordered thought, loss of interest in the outside world and loss of emotional reactions. Bleuler, a Swiss psychiatrist proposed in 1911 that there was not one illness *'dementia praecox'* but rather *'a group of schizophrenias'* and identified what he felt were the primary and secondary symptoms of these disorders (Bleuler 1911). He suggested the primary symptoms were ambivalence,

autism, (loss of interest in the outside world), affective blunting (loss of emotional responses) and altered associations (thought fragmentation). In contrast he felt the secondary symptoms, delusions and hallucinations while often present were not specific to schizophrenia.

1.3.3. Perhaps the most significant subsequent attempt to clarify the clinical definition of schizophrenia was that proposed by Kurt Schneider in 1950 and reflected a shift in emphasis from Bleuler's position. Schneider also regarded certain symptoms as particularly indicative of the disorder, for example particular types of auditory hallucinations and labelled these 'first rank symptoms'. The Schneiderian concept of schizophrenia has remained dominant into early 21st century diagnostic thinking, though it is not without its weaknesses, in particular Schneider's first rank symptoms are neither present in every patients with schizophrenia nor necessarily prove the presence of the disorder(Nordgaard, Arnfred et al. 2008).

1.4. Criticisms of the Concept of Schizophrenia.

1.4.1. The very concept of schizophrenia has remained contentious, perhaps reaching its zenith with the anti-psychiatry movement of the 1960s. Writers such as Thomas Szasz (Szasz 1976; Szasz 1996; Szasz 2008) and R.D. Laing (Laing 1964) viewed schizophrenia as a medical fallacy and more a handy sociological construct serving relatives and the public rather than the needs of the patient. Laing emphasised the role of schizophrenia as a reaction to the demands of modern life and a process that of itself served a therapeutic purpose (Laing 1983). Others postulated that many of the negative features of the illness are in fact a consequence of being labelled with such a stigmatising condition rather than any inherent facet of the disorder itself.

1.5. Symptoms and Signs: How is Schizophrenia Clinically Defined?

1.5.1. Positive Symptoms

1.5.1.1. One of the commonest symptoms (Sartorius, Shapiro et al. 1974) in schizophrenia is hearing sounds or voices, '*auditory hallucinations*' . A hallucination is defined as a perception without a stimulus causing it (*Oyebode* 2008). Hallucinations can occur in any sensory modality though in schizophrenia they are usually auditory. Very often the voices are experienced as personally critical or abusive (Nayani and David 1996).

1.5.1.2. The other major group of positive symptoms are unusual beliefs or '*delusions*'. A delusion is a fixedly held, usually false belief not shared by others from the patient's cultural or social group. The core facet of a delusion is that it is a belief held onto rigidly but without evidence to support it or in the face of evidence to the

contrary. Delusions often develop along very personal themes in schizophrenia, such as persecution, the patient may believe that they are the victim of some form of threat or conspiracy, or of passivity, that their thoughts or actions are being controlled by an external force, though they can develop along any theme, for instance grandiose, sexual, or religious.

1.5.1.3. Many patients also develop *'thought disorder'* manifesting as distorted or illogical speech due to a failure to generate and use language in a logical and coherent fashion. It is typified by a variety of descriptive medical signs including loosening of associations, derailment, tangentiality and knights-move thinking.

1.5.2. Negative Symptoms.

1.5.2.1. '*Negative symptoms*' such as social withdrawal, self-neglect, loss of motivation and initiative, emotional blunting and paucity of speech may seem less troubling to the patient, but often cause families and carers the most distress.

1.5.3. Cognitive Deficits.

1.5.3.1. While not a diagnostic feature of the illness it has been recognised for some time that the majority of patients with schizophrenia experience a variety of cognitive or intellectual difficulties (Reichenberg and Harvey 2007). These range from impaired intelligence to quite specific deficits in for example working memory and executive function. These deficits are often not immediately apparent and their impact on daily living skills or the suitability of psychological or occupational therapy programmes such as cognitive behavioural therapy, can be easily underestimated.

1.5.4. Clinical Subtypes of Schizophrenia

1.5.4.1. Psychiatrists have traditionally recognised several subtypes of schizophrenia depending upon the balance of symptoms present. *'Paranoid'* schizophrenia is typified by the presence of prominent positive symptoms specifically delusions or hallucinations often accompanied by fears of persecution, while the *'Hebephrenic'* subtype is typified by a flattened or incongruous mood, a lack of goal directed behaviour and prominent thought disorder. *'Catatonic'* schizophrenia, now rarely seen in the West is characterised by sustained evidence of abnormal motor behaviour including stupor, excitement, posturing or rigidity. Finally *'Simple'* schizophrenia was said to be associated with a significant loss of personal drive, progressive deepening of negative symptoms and a marked decline in social, academic and employment performance.

1.5.5. Operationalised Diagnosis of Schizophrenia.

1.5.5.1. Two similar international systems are in use to standardise the diagnostic criteria of schizophrenia.

1.5.5.2. The International Statistical Classification of Diseases and Related Health Problems Tenth Revision http://www.who.int/classifications/icd/en/ (World Health Organisation, Geneva (1993)). Its diagnostic criteria for schizophrenia are:

1.5.5.2.1. At least one of the following present most of the time for a month:

- Thought echo, insertion or withdrawal, or thought broadcast;
- Delusions of control referred to body parts, actions or sensations;
- Delusional perception;
- Hallucinatory voices giving a running commentary or discussing the patient or coming from some part of the body;
- Persistent bizarre or culturally inappropriate delusions

1.5.5.2.2. Or at least two of the following present most of the time for a month

- Persistent daily hallucinations accompanied by delusions
- Incoherent or irrelevant speech
- Catatonic behaviour such as stupor or posturing
- Negative symptoms such as marked apathy blunted or incongruous mood

1.5.5.3. The Diagnostic and Statistical Manual Fourth Revision [http://www.dsmivtr.org/ DSM-IV] (American Psychiatric Association) criteria for schizophrenia are:

1.5.5.3.1. Two or more of the following characteristic symptoms present for a significant portion of the time for a month

- Delusions
- Hallucinations
- Disorganised speech
- Disorganised or catatonic behaviour
- Negative symptoms
- Social or occupational decline

1.5.5.3.2. Continuous signs of disturbance for at least six months, but at least one month for the symptoms in criterion A unless treated.

1.5.5.4. By way of contrast, the most commonly reported symptoms of schizophrenia are (Sartorius, Shapiro et al. 1974):

- Lack of insight 97%
- Auditory hallucinations 74%
- Ideas of reference 70%

- Delusions of reference 67%
- Suspiciousness 66%
- Flatness of affect 66%
- Delusional mood 64%
- Delusions of persecution 64%
- Thought alienation 52%
- Thoughts spoken aloud 50%

1.6. Epidemiology & Risk Factors for Schizophrenia.

1.6.1. In spite of schizophrenia's relatively low incidence (15.2 per 100,000)(McGrath, Saha et al. 2004), its prevalence is relatively high (7.2 per 1000)(Saha, Chant et al. 2005), reflecting its tendency to start early in adult life and become chronic.

1.6.2. Schizophrenia typically presents in early adulthood and is rare in childhood(McGrath, Saha et al. 2004). Men are at modestly greater risk of the disorder, have an earlier age of onset than women, and also tend to experience a more severe form of the illness with more negative symptoms, less chance of a full recovery, and a generally worse outcome (Jablensky 2000). Studies have also shown that it is more common in those born in cities, and that the larger the city and the longer the person has lived there, the greater the risk (Pedersen and Mortensen 2001). It seems to be more common in migrants (McGrath 2006; Selten, Blom et al. 2008) despite cultural considerations, though this remains contentious(Selten and Cantor-Graae 2010; Zandi, Havenaar et al. 2010). Some of the most dramatic increases are seen in African and Caribbean people living in the United Kingdom, whose rates are up to 6 times those of the native white population (Fearon, Kirkbride et al. 2006). The rates remain elevated in the children of migrants, but are not reflected in increased rates in their home country (Mahy, Mallett et al. 1999). Environmental and social factors have been particularly implicated in this increased risk, acting through racism and loss of social and family support(Veling, Selten et al. 2007). The risk of schizophrenia in migrants is greatest when they represent a smaller proportion of their local community (Boydell, van Os et al. 2004; Veling, Susser et al. 2008).

1.7. The Causes of Schizophrenia: Genetic and Environmental Factors.

1.7.1. Genetics.

1.7.1.1. Schizophrenia is an aetiologically complex clinical disorder. Its single greatest risk factor is a positive family history(Waddington, Corvin et al. 2007). While

the lifetime risk in the general population in just below 1%, it is 6.5% in the first-degree relatives of patients (Kendler, McGuire et al. 1993), and rises to over 40% in the monozygotic (genetically identical) co-twins of affected patients(Cardno, Marshall et al. 1999). This pattern suggests a link between the genetic proximity of the relative and patient and the risk of developing the illness (Figure 1.1). Furthermore a small but landmark study has suggested that the risk is transmitted equally to the offspring of the well and ill twins from monozygotic discordant pairs, in a manner independent of whether the parent was affected by the illness or not (Fischer 1971).

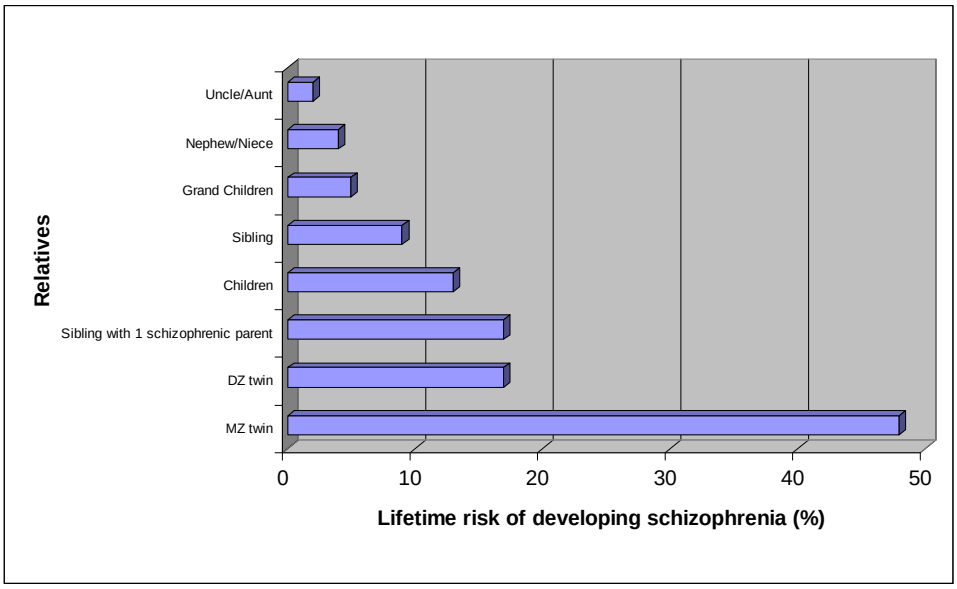


Figure 1.1

Lifetime risk of developing schizophrenia and relationship to proband.

Adapted from: Gottesman II. Schizophrenia Genesis. New York: WH Freeman and Company, 1991.

1.7.1.2. After many years of failure and a multitude of studies, there has been some recent success identifying genes that increase the risk for schizophrenia. In 2002, an Icelandic group identified a haplotype in the Neuregulin 1 (NRG1) gene (Stefansson, Sigurdsson et al. 2002) which appeared to double the risk of illness, a result later replicated in Scotland and Wales, South Africa and China. Other susceptibility genes that have recently emerged include Dysbindin (DTNBP1)(Williams, Preece et al. 2004; Bray, Preece et al. 2005), and DISC1(Porteous, Thomson et al. 2006).

1.7.1.3. It is generally believed that there are many risk genes each of small effect and each relatively common in the general population(Straub and Weinberger 2006; Tandon, Keshavan et al. 2008). Patients probably inherit several risk genes which

interact with each other and the environment to cause schizophrenia once a critical liability threshold has been crossed. In contrast, Crow (Crow 2000) has proposed a genetic model based on a single genetic mutation directly linked to the speciation event that gave rise to Homo Sapiens, cerebral asymmetry and our capacity for language.

1.7.2. The Environment.

1.7.2.1. While concordance rates (the likelihood that both twins are affected) approach 50% in monozygotic twins the fact that they are not 100% implicates environmental factors. A meta analysis has shown that patients with schizophrenia are more likely to have experienced obstetric complications, in particular premature birth, low birth weight and perinatal hypoxia (Canon, Jones et al. 2002). They are also more likely to have been born in late winter and early spring, possibly reflecting intra-uterine viral exposure. These early environmental hazards could act to subtly deviate early brain development.

1.7.2.2. In adulthood different environmental stressors act; associations are seen with social isolation, migrant status, substance misuse and urban life(Boydell, van Os et al. 2004), this remains the case even when life events attributable to the incipient psychosis itself are excluded.

1.7.2.3. Historically certain parenting styles were considered to increase the risk of the illness in children(Frommreichmann 1954; Cheek 1964), however it seems that the way parents raise their children does not have a major impact on future vulnerability. However families do have an important role to play in the course of the illness once it develops(Goulding, Leiner et al. 2008; Seeman 2009); patients with supportive parents have fewer and less severe relapses than those with critical or hostile relatives.

1.7.2.4. Stimulants like cocaine and amphetamines are capable of inducing a picture clinically identical to paranoid schizophrenia, and more recent reports have also implicated cannabis. There is overwhelming evidence that patients with established schizophrenia smoke more cannabis than the general population. Now, well conducted cohort studies like that from Dunedin in New Zealand (Arseneault, Cannon et al. 2002) specifically indicate that early cannabis use, long predating the psychotic symptoms, increases the future risk of schizophrenia four fold while a meta-analysis of prospective studies reported a doubling of this risk. This effect is robust, even after controlling for any self medication effect, undermining the suggestion that this early cannabis use is primarily an attempt to alleviate anxiety or distress actually caused by the developing illness (Henquet, Murray et al. 2005). Of course, only a small proportion of people who use cannabis develop schizophrenia, just as only a proportion of those who misuse alcohol develop cirrhosis. This probably reflects a genetically determined vulnerability

to the environmental agent. Indeed, there have been suggestions that variations in the dopamine metabolising COMT gene influence the vulnerability to develop psychosis in people who use cannabis (Caspi, Moffitt et al. 2005).

1.7.2.5. Collectively these risk factors point to an interaction between biological, psychological and social risk factors driving increasingly deviant development and finally frank psychosis (Howes, McDonald et al. 2004; Broome, Wooley et al. 2005).

1.8. Endophenotypes

1.8.1. Notwithstanding the observations in 1.7.1.2, and that the effects of some genes have been successfully replicated, and that with the advent of new genetic and statistical methods new risk variants are being discovered(O'Donovan, Craddock et al. 2008; Stefansson, Ophoff et al. 2009) most genetic results remain inconsistent and with very small effect sizes(Sklar 2002). One possible explanation for this inconsistency is that the genetic studies have relied on the clinical description of the disorder to define their phenotype, a methodology that may be imperfect for several reasons. Firstly it is clear that even deploying operationalised criteria, the diagnostic margins for schizophrenia are both indistinct and unstable over time (Baca-Garcia, Perez-Rodriguez et al. 2007; Jakobsen, Hansen et al. 2007). Secondly the disorder or syndrome is likely to encompass considerable genetic aetiological and pathophysiological heterogeneity(Craddock, O'Donovan et al. 2007; McClellan, Susser et al. 2007). Thirdly the clinical phenotype is in all probability far removed from the genetic effects that underpin it (Kendler and Neale 2010). One research strategy to address these concerns is to identify and use endophenotypes for clinical schizophrenia, which is in this case the exophenotype.

1.8.2. Endophenotypes are quantitative traits that are hypothesised to lie somewhere on the pathophysiological pathway between genes and illness. By virtue of their quantitative nature they avoid the dichotomy of diagnostic categorisation and hypothetically lie closer to the genetic effects than the disorder they index. Ideally, and hypothetically, they should both be aetiologically less complex and more straight forward to measure, though in practice this may not be the case (Prasad and Keshavan 2008).

1.8.3. Two widely accepted and largely complimentary ‘definitions’ are given below.

1.8.3.1. That an endophenotype is (Gottesman and Gould 2003):

- associated with the illness in the population
- heritable
- primarily state independent

- co-segregates in families
- found in unaffected family members at a higher rate than the general population

1.8.3.2. An intermediate phenotype (often loosely referred to as an endophenotype) is: (Preston and Weinberger 2005)

- a quantitative biological trait that is reliable and reasonably heritable
- associated with variant alleles that distinguish patients and their unaffected siblings from healthy controls on quantitative measures
- reflect a less complex genetic architecture than the disorder

1.8.4. The two terms endophenotype and intermediate phenotype are often used interchangeably. This is in part a product of the relative lack of agreement about the precise definition of the terms and indeed the concept as a whole (see 7.3). To be precise an endophenotype should be reserved for a heritable trait that is not manifestly expressed, it is a mechanistically neutral term, implying no specific link between genes and disorder(Walters and Craddock 2007). By way of contrast an intermediate phenotype is also a heritable trait but with an implied position on the pathophysiological pathway between genes and illness. As will be shown in the course of this thesis the evidence to support this key function of an intermediate phenotype, while desirable, is hard to establish, and thus I will continue to use the more neutral term endophenotype.

1.8.5. An assumption on the endopheotype approach is that the underlying genetic architecture of the endophenotype is less complex that the exophenotype. This too is based on the assumption that the exophenotype is the expression of neural dysfunction in numerous systems, each summarizing the influence of multiple genetic and environmental influences (Gottesman and Gould 2003; Cannon and Keller 2006). The task then is to break down the disorder into its constituent parts, or endophenotypes, that can exist at any number of levels of brain systems (Figure 1.2)

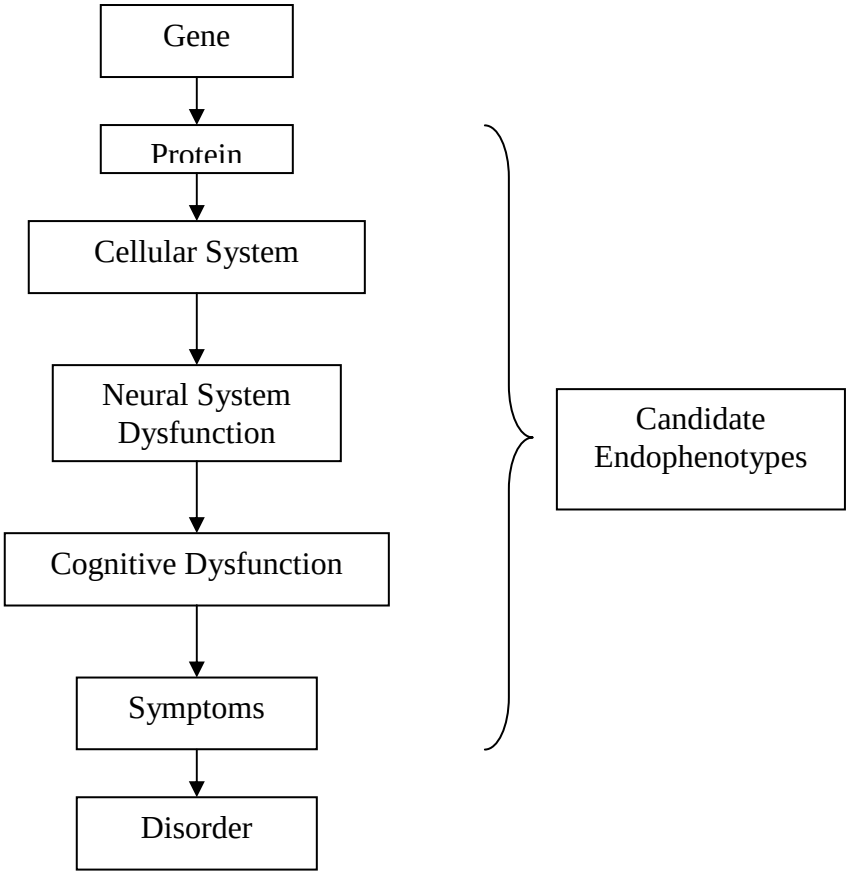


Figure 1.2

Levels of the phenotypic effect from genes to disorder. Candidate endophenotypes can exist at any level indicated. Adapted from Cannon and Keller, 2006.

1.8.6. The primary method used to establish a candidate marker's endophenotypic credentials is the family-study design. In this method patients and their unaffected relatives are contrasted with healthy control subjects to establish that a marker is associated with the disorder, is state independent and is detected at increased rates in the unaffected relatives. While in general the latter is interpreted as evidence of a genetic effect, in fact common environmental factors, shared within families can confound this interpretation. While adoption studies can address this point, in practice the twin method offers the best solution.

1.9. It will be against these endophenotype criteria, particularly those of Gottesman and Gould that I propose to assess four selected candidate markers for schizophrenia in the Maudsely Twin and Family Study of schizophrenia. The selected markers are firstly neurological soft signs, secondly childhood social development and schizotypal personality traits, thirdly brain volume and finally neural function induced by verbal fluency. My aim is to explore how well they meet endophenotype criteria for

schizophrenia.

Chapter 2

2. A Selective Review of Twin Studies in Schizophrenia

2.1. Introduction.

2.1.1. Perhaps one of the few areas of agreement between proponents of the biological and psychodynamic origins of schizophrenia, is that its roots lie within the family (Rosenthal 1960). Indeed Kraepelin's earliest observations of abnormalities in patients' unaffected relatives (Kraepelin 1899) suggested a familial component to this disorder that has only grown in influence.

2.1.2. Familiality however can arise from common (shared within the family) environmental factors, as much as genetic causes. Studies of unaffected relatives alone can not separate these influences. Twin studies offer the only experimental design to separate and quantify genetic from common and unique environmental influences acting on a complex trait. To achieve this and make the most of that opportunity, twin studies should adopt specific statistical techniques and recruit adequately powered samples, two common failings of twin studies in schizophrenia in the past.

2.1.3. This review will explore the nature of twin birth and selectively summarise the results of the twin literature in schizophrenia. I will attempt to highlight where the findings are particularly relevant to the question of endophenotype criteria.

2.1.4. I searched Web of Science and Medline using the terms 'Twin*', 'Twins', 'Multiple birth', 'Schizo*' and 'Schizophrenia'. I tried to access paper copies of papers if they were not available on-line and cross referenced citations as necessary.

2.2. Twin Birth

2.2.1. Twins are the commonest form of multiple births in humans. They are classified as monozygotic (MZ) and dizygotic (DZ), with implied differences in their degree of genetic similarity. The rates of live twin births, both for MZ and DZ twins have increased steadily over the last forty years (Bressers, Eriksson et al. 1987; Imaizumi 2003; Chauhan, Scardo et al. 2010).

2.2.2. DZ twins arise when two fertilised ova implant during a single menstrual cycle. The twins develop in the same pregnancy, but are no more genetically alike than any other sibling pair; thus genetically determined characteristics will vary in DZ pairs in a normal family wise fashion. DZ twins in general, but not exclusively, develop independent chorionic tissues (Fig 2.1), and can experience quite different intra-uterine environments (Denbow and Fisk 1996; Suzuki 2009). Dizygotic twinning rates vary

geographically, seasonally, with maternal age and with family history (Chauhan, Scardo et al. 2010).

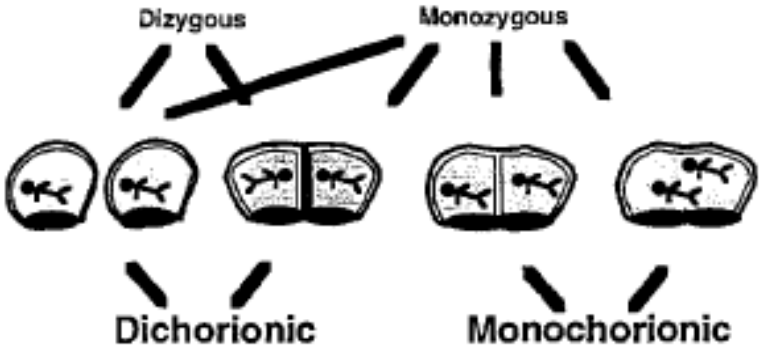


Fig 2.1

The relationship between zygosity and chorionicity

Reprinted from: Denbow, M. L. and N. M. Fisk (1996). "Chorionicity and twins." Current Obstetrics and Gynaecology **6**: 212-219. With permission of Elsevier

2.2.3. MZ twins arise from a single zygote and so share the same genome, as a consequence they tend towards concordance for genetically determined characteristics. The point in gestation at which MZ twinning occurs will determine the degree to which these twins share chorionic and amniotic tissues (Hall 1996) (Fig 2.2). Critically however this does not imply that such twins necessarily share common intra-uterine environments. The clearest example of this is twin-to-twin transfusion syndrome. In this disorder MZ twins, despite, and indeed because, they share a common chorion, can develop vascular anastomses between the two embryonic circulations (Machin, Still et al. 1996; Machin 1996) (Fig 2.2). This leads to one twin being relatively starved of it's vascular supply, while the co-twin is over supplied (Fig 2.3). Both are pathological but 'opposite' states, and act as sources of gross phenotypic discordance (Machin 1996; Slaghekke, Kist et al. 2009; Obladen 2010; Valsky, Eixarch et al. 2010).

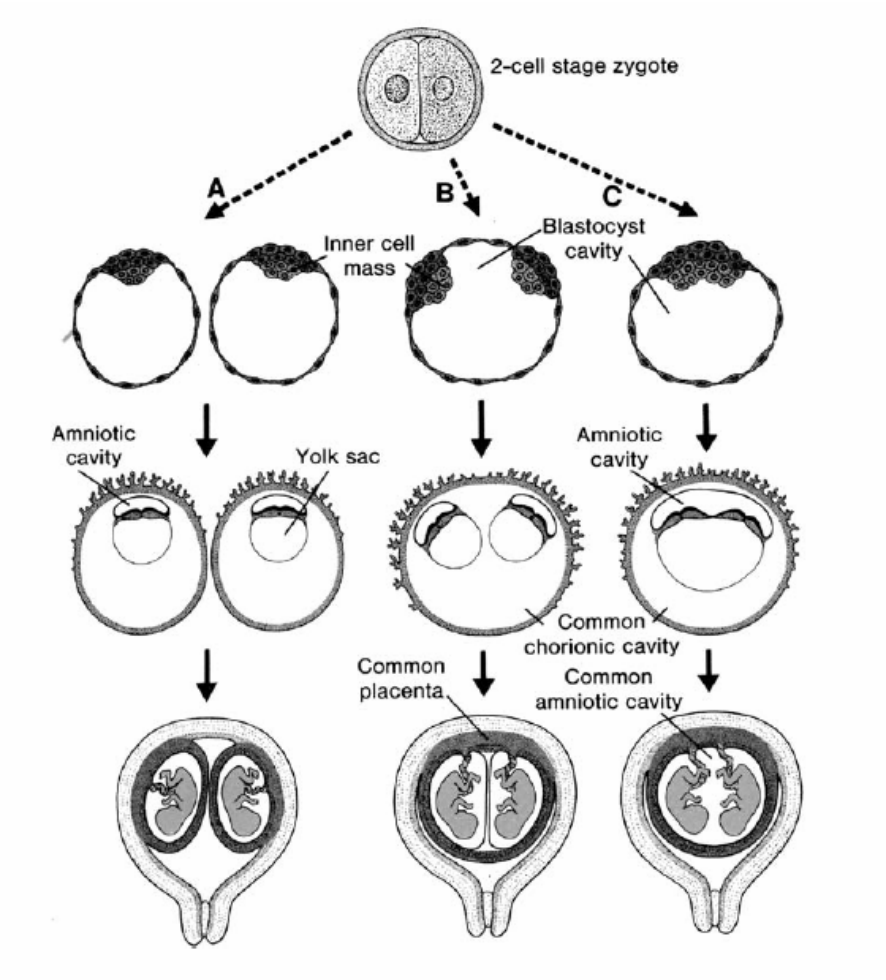


Fig 2.2

The aetiology of monozygotic twins and their relationship with extra-embryonic membranes.

A Split occurs during the late cleavage stage, giving rise to two genetically identical blastocysts. The two embryos each possess a distinct placenta, amniotic cavity and chorionic cavity (dichorionic and diamniotic).

B Splitting of the embryo occurs after implantation, the embryo possesses two distinct inner cell masses. The two embryos share a common placenta, a common chorionic cavity, but have separate amniotic cavities (monochorionic and diamniotic).

C Splitting of the embryo occurs at or shortly before the primitive streak stage. The embryos share a common placenta, a common chorionic cavity and a common amniotic cavity (monochorionic and monoamniotic).

With kind permission from Springer Science+Business Media: Childs Nerv Syst, The embyology of conjoined twins , 20, 2004, 508–525, M.H. Kaufman, figure 1, Originally based on Sadler TW (1985) Langman's medical embryology, 5th edn. Williams & Wilkins, Baltimore.

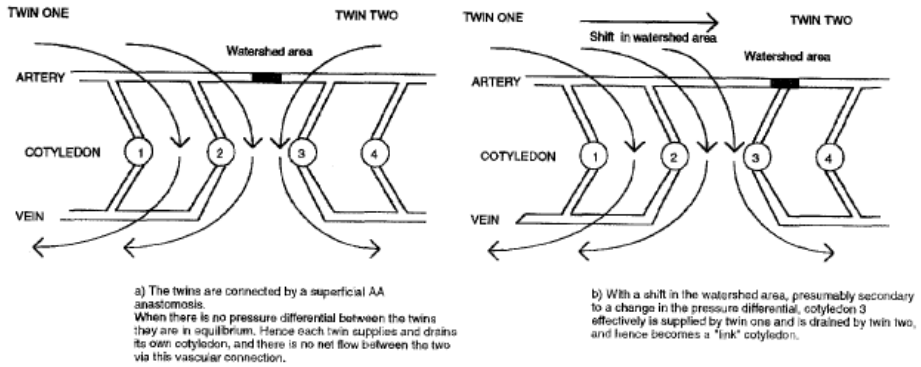


Fig 2.3

The physiological basis of twin-to-twin transfusion syndrome.

A shift in the watershed area between twins within an arterio-arterial anastomosis. It results in the creation of a deep arterio-venous anastomosis.

(Reproduced with permission from *Multiple Pregnancy* (Eds Humphry ward and Martin whittle) published by RCOG Press, 1995. Reprinted from: Denbow, M. L. and N. M. Fisk (1996). "Chorionicity and twins." Current Obstetrics and Gynaecology **6**: 212-219. With permission of Elsevier

2.2.4. The later in gestation that MZ twinning occurs, so the probability of tissue 'sharing' between the two twins increases. The extreme event being a persistent somatic connection between the twins and the phenomenon of conjoining (Kulkarni, Sureshkumar et al. 1994; Kaufman 2004; Spitz 2005).

2.2.5. MZ twinning is an almost uniquely human event and its aetiology is unknown. It is possible that genetic mechanisms, for instance skewed X inactivation (Hall 1996) may be a possible source of the female excess. The lack however of any familial aggregation for MZ twinning suggests that environmental events are more influential (Kaufman 2004). For example the recent increase in MZ twinning rates has been attributed to the increased use of oral contraceptives and assisted conception techniques, while increasing survival figures reflect improvements in obstetric and neonatal care (Chauhan, Scardo et al. 2010).

2.2.6. DZ twinning, and other higher order multiple births, have increased even more dramatically over the same period (Chauhan, Scardo et al. 2010). This increase is principally due to the increased availability and success of assisted conception techniques, particularly hormone induction of ovulation, however other factors such as increasing parental age at conception (Raschka 2000; Malaspina, Harlap et al. 2001), and the increased use of hormonal contraception have probably also played a role.

2.3. Does the Environment Respond to or Create Similarities in Twins?

2.3.1. A core assumption of twin studies is that aetiologically significant environmental factors will not vary systematically between MZ and DZ twins (Plomin, DeFries et al. 1997). The Equal Environments Assumption (EEA) has both its supporters (Kendler, Neale et al. 1993), and detractors (Joseph 1998). If invalid, then any differences in similarity between MZ and DZ twins cannot be attributed to genetic factors alone. While studies comparing the environmental experiences of MZ and DZ twins have reached a broad consensus in terms of their results the interpretations still differ.

2.3.2. The environmental experiences of MZ twins are more similar than DZtwins. Parents report that their MZ twins are more alike on personality and behaviour scores than DZ twins (Jones 1955; Scarr 1968); thus their environment perceives, and indeed expects, them to be more similar. This distinction though is maintained even when parents are mistaken in their assessment of their own twins zygosity, and is consistent with the twins true zygosity.

2.3.3. Subjective and objective ratings of social characteristics and experiences are more similar for MZ compared to DZ adolescent twins (Horwitz, Videon et al. 2003). However parental reports of behavioural, cognitive and personality similarities do not correlate with measures of the twins' physical similarity (Matheny, Wilson et al. 1976; Plomin, Willerman et al. 1976). Furthermore while parental treatment of MZ twins is more similar than DZ twins (Lytton 1977; Kendler, Neale et al. 1994), it has been interpreted as principally determined by behaviours initiated by the twins themselves rather than by their parents. The possible implication of this is that the environmental response is actually an expression of the twins underlying genotype (Scarr and McCartney 1983; Scarr 1996).

2.3.4. Several studies have explored the correlation between perceived zygosity (Kendler, Neale et al. 1993; Kendler, Neale et al. 1994), physical similarity (Hettema, Neale et al. 1995), and social treatment (Morris Yates, Andrews et al. 1990) and phenotypic concordance for major psychiatric illnesses. There was no evidence of a relationship.

2.3.5. Thus while the available evidence shows that the social environment for MZ twins is more similar than DZs, the EEA can not be rejected as this environmental similarity appears itself to be a function of the shared genotype of MZ pairs (Lykken, McGue et al. 1990; Plomin and Bergeman 1991; Plomin and Bergeman 1991; Eaves, Foley et al. 2003).

2.4. MZ Twins: Genetically Similar but Different

2.4.1. The core assumption that MZ twins are genetically identical and that any within pair phenotypic differences can not be genetic in origin is a simplification, at least for some twins (Phillips 1993; Machin 1996; Gringras and Chen 2001; Zwijnenburg, Meijers-Heijboer et al. 2010). There are many examples of genetic discordance in MZ twins. The timing of MZ twinning can influence the degree of chorionic and somatic sharing between MZ twins (2.2.3). Equally the timing of the twinning event can influence the allocation of blastomeres and genetic material between the two developing embryos. These twins will only remain genetically identical if post zygotic genetic, and epi-genetic factors are equal.

2.4.2. MZ twins can differ in their karyotype (Nieuwint, Van Zalen-Sprock et al. 1999), most clearly illustrated by MZ twins discordant for trisomy 21, Down's Syndrome, (Rogers, Voullaire et al. 1982) and Turner's Syndrome, (Uchida, Desa et al. 1983). Further chromosomal differences can occur through chimerism; the presence of genetic material in one individual originating from two. The origins for this lie in the vascular anastomoses between monochorionic MZ twins, which allow stem cell transfer in utero between twins. This also suggests that twin genotyping using non-blood tissue will produce the most accurate uncontaminated results.

2.4.3. While genomic determinism is established at conception, genes are unstable throughout life (Petronis, Vincent et al. 1998). This instability can be expressed at a molecular level, with changes in DNA structure, for instance MZ twins discordant for Fragile X,(Kruyer, Mila et al. 1994), or with epigenetic changes (Petronis and Kennedy 1995; Petronis, Paterson et al. 1999), through alterations in methylation status (Kruyer, Mila et al. 1994; Singh, Murphy et al. 2002), and skewed X inactivation (Orstavik, Tommerup et al. 1995).

2.4.4. Few studies have examined this genetic 'instability' as a potential source of phenotypic discordance for schizophrenia within MZ twins and consequently what little data there is is mixed, reflecting the variety of techniques used and the genetic heterogeneity within the small samples studied.

2.4.5. McDonald (McDonald, Lewis et al. 2003), found no evidence of either genomic or epigenetic discordance in a phenotypically discordant MZ twin pair, while Tsujita (Tsujita, Niikawa et al. 1998), detected differences in genomic DNA within one MZ discordant pair, and attributed this to a mutation in the genomic sequence or methylation status. Friedhoff (Friedhoff, Miller et al. 1995), used subtractive hybridisation to identify novel DNA sequences expressed in one member of an MZ discordant pair. The authors later reported an attempt at validation, detecting the expression of one of the clones in rat cortex and hippocampus, though could not then

replicate their own finding (Zumarraga, Andia et al. 2004). In a similar study, Deb-Rinker et al (Deb-Rinker, Klempan et al. 1999) identified differences in retro-viral related mammalian specific sequences between members of three MZ pairs discordant for schizophrenia and concluded that these sequences, principally expressed in placental tissue, could act as sources of future phenotypic discordance. Vincent et al (Vincent, Kalsi et al. 1998) found evidence of trinucleotide expansion, a possible mechanism of genetic instability, in concordant MZ pairs but not in discordant pairs. In the largest study of its type Nguyen (Lavrentieva, Broude et al. 1999; Nguyen, Bouchard et al. 2003) explored peak similarity scores in MZ discordant and concordant pairs and healthy controls, reporting reduced similarity in the phenotypically discordant pairs, while Polymeropoloulos (Polymeropoulos, Xiao et al. 1993) detected no differences in five MZ discordant pairs.

2.5. Twin Studies of the Heritability of Schizophrenia

2.5.1. Twin studies can only be more widely informative about schizophrenia if twins are not themselves at inherently greater risk of developing the disorder (Jackson 1960; Lidz, Schafer et al. 1962). The majority of studies have found no evidence of this for schizophrenia (Rosenthal 1960; Tienari 1963; Kringlen 1967; Allen and Pollin 1970; Hoffer and Pollin 1970; Nathan and Guttman 1984; Sirugo, Ashenbrenner et al. 2004). However two large national surveys (Klaning, Mortensen et al. 1996; Klaning 1999) did report an increased risk of schizophrenia in DZ twins specifically. Hypothetical explanations for this link have included shared genetic factors, driving both DZ twinning and schizophrenia, though competing hypotheses suggest obstetric complications (Lewis 1996) and increasing parental age could link the two (Crow 1999; Malaspina, Harlap et al. 2001).

2.5.2. Concordance and Heritability

2.5.2.1. Sullivan (Sullivan, Kendler et al. 2003) in a recent meta-analysis of the 12 independent twin studies in schizophrenia, highlighted their methodological heterogeneity, but was still able to report a heritability estimate of over 80% for schizophrenia. They proposed that the most important aetiological influences were additive genetic and, more contentiously, common environmental factors. By way of contrast, two single centre but national studies, used the systematic Finnish twin cohortand the consecutive Maudsley Twin series (Cannon, Kaprio et al. 1998; Cardno, Marshall et al. 1999) and concluded that the most parsimonious, and indeed more widely accepted aetiological model incorporated principally genetic and unique environmental effects.

2.5.2.2. The heterogeneity of the old twin studies may in part reflect their methodological differences. They recruited differently, varying from national population surveys, to studies of psychiatric inpatients, to US Army recruits, each population with their own biases and morbidity levels (Sadrzadeh, Treloar et al. 2001). The diagnostic criteria the studies applied also varied conspicuously, with a potential impact on concordance rates both for schizophrenia (McGuffin, Farmer et al. 1984; Farmer, McGuffin et al. 1987; Franzek and Beckmann 1998) and other disorders (Gatz, Pedersen et al. 2000).

2.5.2.3. Thus 'how' the studies defined schizophrenia had an impact on detected concordance rates. Of phenomenological schizophrenia subtypes, the hebephrenic and 'non-paranoid' subtypes show the greatest genetic influences (Farmer, McGuffin et al. 1984; McGuffin, Farmer et al. 1987; Onstad, Skre et al. 1991; Cardno, Sham et al. 2001) and the strongest tendency to 'breed true', while prototypical Schneiderian schizophrenia (Cardno, Sham et al. 2002) has the lowest heritability and poorly predicts the risk of psychosis in patients' relatives. Crucially, Cardno (Cardno, Sham et al. 2001; Cardno, Rijsdijk et al. 2002) was able to challenge the Kraepelinian dichotomy, in the national Maudsley Twin Series, a study of consecutive admissions to that hospital. The authors reported, for the first time, that schizophrenia, schizoaffective disorder and mania share genetic risk, and noted again that diagnostic criteria influenced concordance rates. They also concluded that the disorganised dimension, analogous to schizophrenia's hebephrenic subtype, was the most genetically determined, with little evidence of a genetic contribution to the positive symptom dimension as a whole.

2.5.3. What are the Other 'Determinants' of Concordance for Schizophrenia?

2.5.3.1. Few studies have reliably identified what subject characteristics, other than zygosity and diagnostic criteria (Kringlen 1967; Kendler and Robinette 1983) influence concordance rates. Rosenthal's (Rosenthal 1959) reanalysis of Slater's original twin data (Slater 1953) suggested concordant pairs were more likely to come from multiply affected families, and speculated that concordant pairs had received a greater genetic load. This hypothesis lead others to suggest that discordant pairs actually harboured very little genetic risk (Luxenburger 1940). However the experimental data to address this is restricted to evidence that MZ discordant pairs are subject to greater environmental stress in the form of obstetric complications (Cantor-Graae, McNeil et al. 1994; Cantor-Graae, McNeil et al. 1994; Van Oel and Kahn 1997; Kunugi, Tsukue et al. 1999; Van Oel, Baare et al. 1999). Arguing against this point, there is no evidence that the offspring of discordant twins, whether from the patient or the unaffected co-twin, were at any less risk of developing schizophrenia than their concordant counterparts Kringlen (Kringlen and Cramer 1989).

2.5.3.2. There is some data to suggest that concordant pairs develop schizophrenia at a younger age, with age at onset highly correlated within pairs (Allan, Cardno et al. 2009). They ,may have a more extreme symptom profile, reflecting a more severe form of the illness, though these findings are inconsistent (Slater 1953; Kringlen 1967; Fischer 1973; McGuffin, Asherson et al. 1994; Allan, Cardno et al. 2009). Finally it is possible that male gender might inflate concordance rates (Kringlen 1967; Samuels 1978; Samuels 1979).

2.6. Is the Familial Risk for Schizophrenia Specific?

2.6.1. While twin and family studies suggest that an individual's proximity to the proband determines their risk of developing schizophrenia, it remains to be seen whether this risk can manifest itself in other non-schizophrenia spectrum disorders. A question formalised as whether the genetic risk for schizophrenia leads to an increased liability to (Kendler and Diehl 1993):

- schizophrenia alone
- schizophrenia and all schizophrenia spectrum disorders
- all non-affective psychoses
- a broad liability to all psychiatric illness

2.6.2. Two related reports from the Vietnam Era Twin Registry (Lyons, Toomey et al. 1997; Lyons, Bar et al. 2002) have shown that schizophrenia is associated with a greater risk of affective and anxiety disorders in probands, but that this does not extend to their well co-twins, in contrast to nicotine dependence which did.

2.6.3. DiLalla (Dilalla and Gottesman 1995), in a highly selected discordant twin sample reported normal personality development in the well co-twins.

2.6.4. By contrast many other studies have demonstrated increased rates of personality disorder (Kendler and Robinette 1983; Torgersen, Onstad et al. 1993), abnormal communication styles (Docherty and Gottesman 2000), anxiety symptoms (Kendler and Robinette 1983; Torgersen, Onstad et al. 1993; Argyropoulos, Landau et al. 2008), and affective disorders (Argyropoulos, Landau et al. 2008), in the well co-twins of discordant MZ pairs, with some evidence of a risk gradient between MZ and DZ co-twins (Kendler and Robinette 1983). This if true, suggests that the familial risk for schizophrenia increases risk to a broad spectrum of other psychopathology in 'unaffected family members'. This observation is consistent with the current concept of the At Risk Mental State (Yung, Phillips et al. 1998; Phillips, Yung et al. 2002; Yung, Phillips et al. 2004; Nelson, Yung et al. 2008) and the often very non-specific early psychiatric presentation of psychosis and schizophrenia. Finally Reichenberg (Reichenberg, Weiser

et al. 1999; Reichenberg, Rabinowitz et al. 2000), in a unique but small sample assessed behavioural and psychological functioning in adolescent MZ twins, before one twin later developed schizophrenia. They detected the greatest deficits in the future probands, but noted that the well co-twins expressed deficits in social functioning, well in advance of their co-twin ever being ill.

2.6.5. Thus it seems that the schizophrenia genotype does not exclusively breed true. This raises the possibility that some of the familial, and perhaps genetic, risk for schizophrenia increases vulnerability to a wider range of psychopathology, while perhaps others aspects of the risk are much more specific to the vulnerability for psychosis or schizophrenia itself.

2.6.6. A complimentary research strategy focuses on exploring the vulnerability to non-schizophrenia psychopathology in the children of MZ discordant twins. The strength of this design is the assumption that the well co-twins represents the 'purest' form of unaffected obligate carrier, its weakness the limited statistical power of often incredibly small samples. Fisher's landmark study (Fischer 1971), contentiously revisited (Gottesman and Bertelsen 1989; Torrey 1990), suggested that well co-twins were indeed at high genetic risk, and actively transmitted that risk to their own offspring, which in turn bred true. In contrast Kringlen (Kringlen and Cramer 1989), in a larger and methodologically superior but still limited study, detected statistically increased rates of broadly defined schizophrenia spectrum disorders in probands' children compared to the well co-twins' children.

2.7. When do Discordant Pairs Diverge?

2.7.1. Many studies (Arieti 1949; Pollin, Stabenau et al. 1965; Pollin, Stabenau et al. 1966; Kringlen 1967; Stabenau and Pollin 1967; Mosher, Pollin et al. 1971; Fischer 1973; Kunugi, Urushibara et al. 2003) have demonstrated that probands from discordant pairs manifest abnormalities of personality and non-psychotic symptomatology in early childhood, and well before any psychotic symptoms emerge, with increased levels of passivity, dependence and anxiety. These abnormalities are maintained into adult life with increased emotionality, labile mood, paranoia and anxiety.

2.7.2. Fuller Torrey (Fuller Torry, Bowler et al. 1994) has argued that developmental discordance is not a uniform phenomenon within MZ discordant twins, but rather that a subgroup of MZ discordant pairs diverge relatively early in life, around the age of 5, with a second peak of discordance later, and relatively closer to the onset of frank psychotic symptoms in the probands. While the early divergent group are subsequently no more severely affected by the illness, they do tend to be male, with more obvious risk hsitories, though this can be for family history or obstetric complications. In

perhaps the most methodologically robust study, van Oel et al retrospectively examined adult discordant twins' school reports. They found that elementary school performance differentiated twins who only clinically diverged many years later (van Oel, Baare et al. 2001).

2.8. Environmental Mechanisms and Measures in Schizophrenia

2.8.1. Dermatoglyphics

2.8.1.1. Dermatoglyphic abnormalities hypothetically offer a time sensitive marker of ectodermal development. Studies in twins with schizophrenia have tended to be small and underpowered.

2.8.1.2. Bracha (Bracha, Torrey et al. 1991) found evidence of abnormal development early in the second trimester of pregnancy in probands with schizophrenia, and concluded that this was evidence of an aetiologically important environmental agent acting at that developmental stage. All but one of several other studies (Bracha, Torrey et al. 1992; Davis and Bracha 1996; van Os, Fananas et al. 1997; van Oel, Baare et al. 2001; Kelly, Cotter et al. 2004) reported reduced a-b ridge count correlations within discordant compared to control pairs, though only two (Davis and Bracha 1996; Kelly, Cotter et al. 2004) actually detected greater numbers of abnormalities in the probands themselves. Thus they could provided only limited evidence to support their specificity for schizophrenia. While the two of these studies that assessed finger ridge fluctuating asymmetry (FA) in psychotic twins reached strikingly different conclusions. The failure of these studies to detect significant differences between the probands and their well co-twins appears to undermine their usefulness as an index of aetiologically relevant unique environmental insult.

2.8.1.3. Two studies assessed ridge dissociation in twins, (van Os, Fananas et al. 1997; Rosa, Fananas et al. 2000), both detected more abnormalities in the patients. However one (van Os, Fananas et al. 1997) detected greater rates in concordant compared to discordant pairs, while the other (Rosa, Fananas et al. 2000), detected more abnormalities in probands compared to their well co-twins in discordant pairs.

2.8.1.4. The evidence from twin studies of ectodermal development in psychosis is thus mixed. There is some dermatoglyphic evidence to suggest the presence of early developmental divergence in MZ pairs who later become discordant for schizophrenia, and that this divergence may be present from as early as the first 15 weeks of life in utero. However the presence of these markers, albeit at attenuated levels in the well co-twins as well, necessarily undermines their validity as markers of the unique environmental insult responsible for schizophrenia. It seems more likely that these

markers reflect abnormal ectodermal development in utero, but that they give little indication of the underlying aetiology.

2.8.2. Obstetric Complications

2.8.2.1. Obstetric complications (OCs), in particular perinatal hypoxia, are themselves the environmental risk factors. Notwithstanding that they are almost always retrospectively assessed, frequently 'shared' within twin pairs, and that twins, as with all multiple pregnancies are inherently at greater risk of OCs, including prematurity and low birth weight (Chauhan, Scardo et al. 2010), several attempts have been made to explore their association with schizophrenia in twins.

2.8.2.2. Early evidence suggested that OCs were over-represented in discordant twins. Two small highly selected cohorts (Lewis, Chitkara et al. 1987; Fuller Torry, Bowler et al. 1994) detected birth weight discordance in discordant pairs, with the lighter twin more likely to develop schizophrenia. Some (McNeil, Cantorgraae et al. 1994; McNeil, Cantorgraae et al. 1994) have reported a reducing gradient of peri and neonatal complications in MZ discordant through MZ concordant to MZ control pairs. However others (Kringlen 1967; Fischer 1973; Onstad, Skre et al. 1992) could not replicate these findings, nor detect any evidence of an increased risk of OCs in MZ discordant and concordant pairs at all.

2.8.2.3. Gilmore (Gilmore, Perkins et al. 1996) used ultrasound and reported greater mean parietal diameter differences in MZ than DZ pairs, and suggested that environmental factors, acting through the placenta had caused divergent brain development, in a portion of the MZ twins. The same NIMH sample (Cantor-Graae, McNeil et al. 1994) demonstrated an association between early pregnancy complications and minor physical anomalies in the MZ discordant group, though these were detected both in the probands and the well co-twins. The study also used unvalidated makers of chorionicity to suggest that the environmental agent may have been infectious (Davis and Phelps 1995; Davis, Phelps et al. 1995). A related study (McNeil, Cantor-Graae et al. 2000) explored the link between obstetric complications and brain structure, reporting reduced hippocampal volume and increased lateral ventricular volume with increased OCs. However this was in the discordant group as a whole, not just the patients. It also reported that the length of labour predicted hippocampal volume, and raised the suggestion that a complicated delivery, through hypoxia, might cause brain changes in regions implicated in the pathophysiology of schizophrenia.

2.9. Neuropsychology

2.9.1. More than any other field, with the exception perhaps of structural neuroimaging, twin neuropsychological studies have evolved from deficit detection, through estimation of their heritability, to the identification and quantification of shared genetic risk with schizophrenia, thereby establishing their endophenotypic credentials.

2.9.2. Goldberg (Goldberg, Ragland et al. 1990; Goldberg, Torrey et al. 1993; Goldberg, Torrey et al. 1995) originally reported in the NIMH twins, that probands from MZ discordant pairs under-performed compared to their unaffected co-twins on vigilance, concept formation, learning and memory. They reported only limited genetic effects (Goldberg, Torrey et al. 1995) in executive function, implicating unique environmental factors in determining the patients' deficits in that domain. Subsequently, Reichenberg (Reichenberg, Rabinowitz et al. 2000) examined premorbid cognitive function in a national twin cohort and detected evidence of familial deficits in some aspects of cognition related to schizophrenia. The twins who later developed schizophrenia scored worse than controls, with no significant differences in premorbid performance within the discordant pairs.

2.9.3. Two studies contrasted MZ and DZ discordant pairs (Docherty and Gottesman 2000; Pardo, Knesevich et al. 2000) on specific cognitive tasks. They reported a genetic contribution to perseverative errors (Pardo, Knesevich et al. 2000) and intrusive missing information (Docherty and Gottesman 2000), finding evidence for genetic effects, with greater deficits in the unaffected co-twins from MZ than DZ pairs.

2.9.4. Cannon (Cannon, Huttunen et al. 2000) in a large national cohort used a structured cognitive battery to establish the heritability of neuro-cognitive deficits in schizophrenia. They concluded that spatial working memory, divided attention, intrusions during recall and choice reaction time were linked to the genetic load for the illness, and that spatial working memory and recall intrusions were genetically determined. In contrast, verbal and visual episodic memory, more impaired in the ill than the well MZ twins, suggested unique environmental effects. The same group (Johnson, Huttunen et al. 2003; Johnson, Tuulio-Henriksson et al. 2003) later reported that cognitive function was mediated in those at genetic risk by schizotypal traits, and that in the absence of schizotypy there were few cognitive deficits. The authors suggested that future genetic studies should adopt such a bivariate phenotype.

2.9.5. Most recently Toulopoulou et al first in a large carefully phenotyped psychotic twin series (Toulopoulou, Picchioni et al. 2007), then in an international multi site collaboration(Toulopoulou, Goldberg et al. 2010) used full genetic modelling techniques to highlight the significant correlation between intelligence and working memory, and schizophrenia and most importantly that shared genetic variance accounted

for a large proportion of the covariance between the two. Recently selected executive function deficits, including verbal fluency performance showed similar results (Owens, Rijsdijk et al. In Press).

2.9.6. Thus twin studies of cognition in schizophrenia have suggested that specific cognitive markers may be able to act as sensitive markers of the genetic risk for schizophrenia, with particular emphasis on intelligence, working memory and divided attention, executive function, intrusions during recall, and choice reaction time to visual targets, as the most likely targets for further investigation.

2.10. Neuroimaging

2.10.1. Whole Brain Volume

2.10.1.1. Structural imaging studies of twins with schizophrenia have in the main been hampered by their relatively small sample sizes and limited statistical power. Only one has been sufficiently powered to implement full genetic modelling, and so to tease apart genetic and common environmental influences on the structures being investiged.

2.10.1.2. Two qualitative studies (Suddath, Christison et al. 1990; Noga, Bartley et al. 1996), used overlapping MZ discordant cohorts, but failed to agree on the presence of significant differences between patients and their well co-twins. Two later studies, in the same sample (Narr, Cannon et al. 2002; Narr, van Erp et al. 2002), reported no differences in intracranial, grey or white matter volume between patients and either their healthy co-twins or controls.

2.10.1.3. In contrast, later larger studies, including up to 15 discordant pairs, have shown reduced whole brain volume (WBV) in discordant pairs compared to controls (Baare, van Oel et al. 2001; Hulshoff Pol, Brans et al. 2004), and that within these pairs, patients had lower WBV than their well co-twins (Noga, Bartley et al. 1996; van Haren, Picchioni et al. 2004). These results suggested in the main familial effects, acting through genetic or common environmental factors, with less compelling evidence for additional unique environmental effects driving further volume loss in the patients. This finding was developed further by Rijsdijk et al (Rijsdijk, Van Haren et al. 2005), who reported on a large combined Maudsley Family and Twin Schizophrenia sample. The authors used genetic model fitting and were able to report firstly that whole brain volume in schizophrenia was heritable, but secondly and perhaps more importantly in this context, that there was evidence of a significant genetic correlation with schizophrenia, thus that the genes that cause or are linked to schizophrenia, also reduce whole brain volume.

2.10.1.4. When grey and white matter volumes are segmented (Hulshoff Pol, Brans et al. 2004), white matter reductions were detected in the discordant group as a whole,

suggesting genetic or common environmental effects, with the grey matter deficits appearing to be more specific to the probands, implying unique environmental effects on that tissue class. Intra-class correlation coefficients (ICCs) for whole brain, grey and white matter volumes (Baare, van Oel et al. 2001; Narr, Cannon et al. 2002; Hulshoff Pol, Brans et al. 2004; van Haren, Picchioni et al. 2004) have been consistently greater in MZ compared to DZ twins, surprisingly irrespective of their concordance for schizophrenia, and consistent with the data from healthy control twins, that prominent genetic effects act on all these volumes.

2.10.1.5. The most sophisticated image analysis methods applied so far to cortical grey matter in discordant twins (Cannon, Thompson et al. 2002) used a novel mapping algorithm to produce estimates of the location and magnitude of illness/unshared environmental and genetically driven deficits over the entire cortical surface in schizophrenia. Non-genetic effects were found particularly in the dorso-lateral prefrontal cortex, superior and middle temporal gyrus, parietal association and motor cortex, with contrast genetic effects particularly localised to a region in frontal cortex.

2.10.1.6. Brans et al (Brans, Van Haren et al. 2007) utilised a twin modelling approach in a unique longitudinal study that rescanned many of the original Utrecht twins (Baare, van Oel et al. 2001), approximately five years after their original scan. This rigorous approach has resulted in the only MRI follow up study in twins in schizophrenia and suggested that accelerated loss of brain volume was a feature both of schizophrenia and its genetic risk, and that the genetic correlation, for example with loss of whole brain volume, was over 60%.

2.10.1.7. Taken as a whole, the majority of these results strongly suggest that brain volumes in health and in schizophrenia are highly heritable. Furthermore that familial mechanisms, central to schizophrenia have an impact reducing total cerebral, grey and possibly white matter volumes. More contentiously these factors, again acting through genetic risk, then contribute to accelerated loss of tissue volume over the lifespan.

2.10.2. Region Of Interest

2.10.2.1. Region of interest studies can help to address the anatomical specificity of the global tissue loss described above.

2.10.2.2. In MZ discordant twin pairs, lateral ventricular volume is greater in patients than their healthy co-twins, controls (Reveley, Reveley et al. 1982; Reveley, Reveley et al. 1984; Ohara, Xu et al. 1998), and more tentatively than patients from concordant pairs, (van Haren, Picchioni et al. 2004), suggesting a greater sensitivity to environmental insult in the patients from those pairs. However Styner et al (Styner, Lieberman et al. 2005) have shown that despite the presence of the illness, lateral

ventricular volume and shape can remain highly conserved, even within MZ discordant pairs, suggesting that strong genetic or common environmental influences continue to act on this structure.

2.10.2.3. Only two studies have assessed brain volumes in relation to environmental agents in twins with schizophrenia. The first (Reveley, Reveley et al. 1984) found a relationship between obstetric complications (environmental risk) and increased lateral ventricular volume, but only in healthy controls, and that a positive family history for schizophrenia (and so more 'genetic' risk) was associated with smaller lateral ventricular volumes in patients. The second (McNeil, Cantor-Graae et al. 2000) reported that perinatal complications were associated with smaller hippocampi and larger lateral ventricular volumes in patients. These results suggest that obstetric complications, and in particular perinatal hypoxia, may represent an environmental insult, that in genetically vulnerable individuals, could cause tissue loss, smaller hippocampi and ventricular enlargement, while genetic risk alone may lead to more subtle structural changes.

2.10.2.4. Medial temporal lobe structural differences have been detected in both members of discordant pairs compared to controls (Baare, van Oel et al. 2001; Narr, van Erp et al. 2002), but with no additional deficits in the patients, while others (Suddath, Christison et al. 1990; McNeil, Cantor-Graae et al. 2000; van Erp, Saleh et al. 2004) have found further illness specific volume loss in the patients from MZ discordant pairs, and that these deficits were correlated with cognitive performance, including verbal working memory (Goldberg, Torrey et al. 1994).

2.10.2.5. The only study so far that attempted to assess hippocampal sub-regions reported that volume loss was greatest posteriorly (Narr, van Erp et al. 2002). They also applied genetic model fitting techniques (van Erp, Saleh et al. 2004) and reported lower heritability estimates for hippocampal volume in discordant twins (42%) than controls (71%).

2.10.2.6. Two studies (Casanova, Sanders et al. 1990; Narr, Cannon et al. 2002) addressed the corpus callosum in MZ discordant pairs. Both reported no evidence of abnormalities in callosal length or area but found upwards displacement in probands. The later study though also reported this effect in the unaffected co-twins, and suggested a genetic or common environmental effect in schizophrenia, possibly driving or a response to changes in lateral ventricular volume or shape.

2.10.2.7. Two studies focused on the thalamus. The first and smaller study (Bridle, Pantelis et al. 2002) reported no significant differences between controls and MZ discordant or concordant pairs for thalamic volume, while probands had larger caudate lobes than their co-twins, but not controls. In the second and larger study Ettinger et al

(Ettinger, Picchioni et al. 2007) detected reduced thalamic volume in MZ concordant pairs compared to controls, with a gradient from MZ healthy controls, through MZ discordant to the MZ concordant pairs. Contentiously this was interpreted as evidence of a sliding scale of genetically driven volume loss across these groups.

2.10.3. Voxel Based Morphometry

2.10.3.1. Only two studies have so far used a whole brain voxel based approach in twins with schizophrenia. Both the Utrecht and Maudsley studies examined samples that overlapped with their respective whole brain studies described above (Baare, van Oel et al. 2001; van Haren, Picchioni et al. 2004). Neither were sufficiently powered to implement full genetic modelling or estimate heritability, and were only able to use regression to detect group effects.

2.10.3.2. In the first (Hulshoff Pol, Schnack et al. 2006) despite the earlier report of global grey matter volume deficits in patients, there were no regional deficits that discriminated patients from their unaffected co-twins. There were however significant difference in the left orbitofrontal gyrus between the discordant twins as a whole and controls.

2.10.3.3. The second study (Borgwardt, Picchioni et al. In Press), did not include DZ twins but did include MZ concordant pairs. The authors detected no differences between twins with schizophrenia from concordant and discordant pairs. They also found no evidence of focal grey matter deficits between the MZ discordant well and the healthy control twins. However unique environmental differences were detected in the insula and the superior temporal gyrus, as well as the cingulate gyrus in the twins with schizophrenia.

2.10.4. Functional Imaging

2.10.4.1. Despite a wealth of functional imaging studies in schizophrenia very few have focused on twins. What few studies there are, are extremely heterogeneous, so that few firm conclusions can be drawn.

2.10.4.2. One functional MRI study examined lateralisation for word tasks in MZ discordant and control twins (Sommer, Ramsey et al. 2004). It reported an increase in activity in right sided language regions, in both members of discordant pairs, and interpreted this as evidence of genetically determined abnormal language lateralisation. In contrast, a case report of a single MZ discordant pair (Spaniel, Hajek et al. 2003), highlighted the non-genetic nature of a very similar deficit detected only in the patient, with no impairment in the well co-twin. The authors linked the patient's deficit to abnormal hippocampal function subsequently detected at spectroscopy. The same group

(Spaniel, Herynek et al. 2005) reported further illness specific deficits in the structural integrity of the globus pallidus detected using MR relaxometry.

2.10.4.3. A larger study (Berman, Torrey et al. 1992) used radio labelled Xenon to measure regional cerebral blood flow and reported localised frontal, but no temporal, lobe deficits in patients from MZ discordant pairs, in response to a frontal lobe challenge. They detected no functional deficits in the well co-twins. These data suggest that the frontal lobe deficits were illness specific and reflected the impact of unique environmental factors. In a secondary analysis the same authors (Weinberger, Berman et al. 1992) demonstrated that within pair differences in hippocampal volume, measured using structural MRI predicted these frontal lobe blood flow deficits, and interpreted this as evidence of illness specific (environmental) abnormalities in a distributed cortical-subcortical network.

2.10.4.4. In contrast a later study (Hirvonen, van Erp et al. 2005), examined only the well co-twins from MZ and DZ discordant pairs. It detected increases in caudate D2 receptor density correlated with genetic risk.

2.10.4.5. Finally one single proton magnetic resonance spectroscopy study in a modest mixed zygosity sub sample of the Finnish twin cohort (Lutkenhoff, van Erp et al. 2010), detected evidence of genetically determined prefrontal cortical glutamate reductions, with additional disease specific elevations in hippocampal N-acetylaspartate, creatine and glycerophosphocholine. This preliminary data supported a model of genetically determined glutamate dysfunction in schizophrenia.

2.11. Other Biological Markers

2.11.1. Electrophysiology

2.11.1.1. To date five studies have examined electrophysiological function in MZ twins with schizophrenia (Stassen, Coppola et al. 1999; Weisbrod, Hill et al. 1999; Bachman, Kim et al. 2003; Weisbrod, Hill et al. 2004; Hall, Rijsdijk et al. 2007) all with modest sample sizes.

2.11.1.2. Weisbrod (Weisbrod, Hill et al. 1999) reported reduced P300 amplitude in both patients and their well co-twins in MZ discordant pairs and concluded that this reflected the genetically determined vulnerability to schizophrenia. Two other studies though, one by the same group (Stassen, Coppola et al. 1999; Weisbrod, Hill et al. 2004), produced contrasting results. The later studies noted that pairs with at least one ill member had lower within-pair EEG concordance, and concluded that this was driven by unique environmental effects. Furthermore both studies, identified EEG parameters that could reliably distinguish the patients from their unaffected co-twins, suggesting its possible use as a disease specific marker.

2.11.1.3. Four other studies have used different cognitive tasks to manipulate the EEG signal in twins (Bachman, Kim et al. 2003; Ahveninen, Jaaskelainen et al. 2006; Hall, Rijsdijk et al. 2007; Bachman, Kim et al. 2008). They all elicited what they interpreted as electrophysiological correlates of the genetic risk for schizophrenia. However only the latter (Hall, Rijsdijk et al. 2007) was able to quantify the heritability, phenotypic and genetic correlation of its selected electrophysiological markers with schizophrenia, culminating, using those indeces in its claim that P50 suppression was the best candidate endophenotype marker for schizophrenia.

2.11.1.4. Thus while much of the earlier work is mixed, the emerging evidence from the newest electrophysiological studies, that have included the largest, but still modest subject numbers, is that selected electrophysiological measures offer scope as candidate endophenotype markers, though this needs replication.

2.11.2. Eye movements

2.11.2.1. Two Scandinavian studies examined eye tracking abnormalities between MZ and DZ discordant pairs, though did not compare within twin pairs, nor include any control twins. They both detected smooth pursuit abnormalities in the well co-twins and noted that MZ pairs were more similar than DZ pairs whether assessed qualitatively (Holzman, Kringlen et al. 1978; Holzman, Kringlen et al. 1980), or quantitatively (Holzman, Kringlen et al. 1977; Holzman, Kringlen et al. 1980). The authors concluded that eye-tracking deficits could serve as genetic markers for schizophrenia as they were familial and probably genetically determined, finally suggesting also that these deficits were more 'penetrant' than schizophrenia's clinical phenotype.

2.11.2.2. Two studies (Torrey, Gottesman et al. 1994; Litman, Torrey et al. 1997) contentiously failed to reproduce these results (Holzman, Levy et al. 1997). The later in particular used more advanced recording techniques, but adopted a different statistical model, that could have contributed to this inconsistency. Both however found that the well co-twins were not significantly impaired compared to controls, and rejected a genetic model for smooth pursuit abnormalities in schizophrenia.

2.11.2.3. Most recently, in the Maudsley cohort (Ettinger, Picchioni et al. 2006), contrasted relatively modest MZ discordant and control twin samples, and produced evidence of genetically determined deficits in antisaccade gain and latency that were consistent with familial effects.

2.11.3. Neurological Soft Signs

2.11.3.1. Mosher (Mosher, Pollin et al. 1971), studied MZ discordant pairs and detected an increase in neurological soft signs in probands but not in their co-twins,

while Kelly (Kelly, Cotter et al. 2004) could not detect any difference between probands and their well co-twins. In contrast two other studies (Rosenthal and Kety 1968; Torrey, Gottesman et al. 1994), reported that the well co-twins from MZ discordant pairs occupied a position between probands and healthy controls, suggesting both genetic and unique environmental effects driving the neurological soft signs. Obstetric complications have been suggested to be one possible source of this environmental risk in twins (Mosher, Pollin et al. 1971; Cantorgraae, McNeil et al. 1994).

2.11.3.2. Three studies (Boklage 1977; Luchins, Pollin et al. 1980; Lewis, Chitkara et al. 1989) have explored laterality and handedness (Crow 1999) in twins with schizophrenia. The evidence is mixed but suggests that in twin pairs with at least one left handed member, the proband is more likely to be left handed, though none of the studies were able to explore the aetiology of this further.

2.11.4. Other Markers

2.11.4.1. One study (Torrey, Gottesman et al. 1994) assessed minor physical anomalies (MPAs) in MZ discordant, concordant and control twins but detected no significant increase in the patients with MPAs highly correlated even within discordant twin pairs.

2.11.4.2. Very few studies have measured peripheral biological markers in twins with schizophrenia. Vander Putten (Vander Putten, Fuller Torry et al. 1996) detected significantly greater differences in plasma proteins in MZ discordant than control pairs, and concluded these reflected non-genetic factors. Two (Stabenau, Pollin et al. 1969; Poltorak, Wright et al. 1997) examined blood and CSF markers, and reported abnormal rates in the patients compared both to their well co-twins and controls. Again these were interpreted as evidence of unique environmental effects.

2.11.4.3. Kopola (Kopala, Good et al. 1998) used a smell identification task in the NIMH twin sample. They reported that MZ discordant twins as a whole experienced deficits, but were insufficiently powered to detect differences between the well co-twins and either the patients or the controls. This suggested a familial defect in cerebral function, detected through impaired smell identification, but that additional illness specific effects, (unrelated to smoking) were also having an effect.

2.11.5. Medication Response

2.11.5.1. Two twin case reports have reported evidence supporting genetic determination of neurotransmitter systems predicting antipsychotic medication response in schizophrneia. They each reported on pairs of MZ twins concordant for schizophrenia who shared their clinical response to Clozapine (Vojvoda, Grimmell et al. 1996) and olanzapine (Mata, Madoz et al. 2001). Despite the obvious limitations of size, these case

reports highlight the huge potential of individualised treatment through pharmacogenetics.

2.12. Summary

2.12.1. Twin studies have over many years produced influential and significant results that have steered thought about the aetiology and pathophysiology of schizophrenia. From the early concordance studies to current advanced genetic modelling this remains the case. Over the last decade the methodology underpinning twin research in schizophrenia has advanced considerably and sample sizes have slowly increased.

2.12.2. The early generally small and highly selected twin studies reliably established the presence of a multitude of deficits in the patients with schizophrenia and their co-twins. This emphasised the familiarity of the disorder. Qualitatively similar but quantitatively less marked deficits, for many of these traits, were present in the MZ and DZ unaffected co-twins further supporting a familial effect. These familial influences though could arise from genetic and shared familial environmental influences. It is only a small number of these studies that tried to and were able to detect either a 'gradient' effect from the DZ to MZ unaffected co-twins or greater correlations in the MZ than DZ samples, that begin to establish specific evidence for genetic effects. Thus the majority of the published twin literature can only describe their markers on a limited number of the endophenotype criteria.

2.12.3. Many of these studies can be criticised for:

- often focussing exclusively on MZ discordant and MZ control samples
- failing to deal appropriately with the familial nature of the data and the lack of independence of observations between members of the same twin pair. Many studies either ignored this statistical consideration, or dropped one member at random from each pair
- failing to recruit samples with sufficient statistical power
- failing to employ genetic modelling techniques to discriminate between and quantify the genetic and shared environmental effects on the traits underinvestigation.

2.12.4. It is only relatively recently with the emergence of larger national and indeed international twins studies, that have recruited sufficiently large samples, and with the ability to deploy the most suitable statistical and genetic modelling techniques, that we have started to be able to quantify the genetic links between these familial deficits and schizophrenia.

2.12.5. At present there are two linked international collaborations underway, the Schizophrenia Twin and Family (STaR) consortium involving centres in Utrecht, Jena,

Helsinki and London, and the EUTwins Network, sponsored by the European Union through a Marie Curie Training Network grant that extends to nine European centres. Such international collaborations offer the best means of evaluating the suitability and usefulness of candidate markers as endophenotypes of schizophrenia. It is only by applying the most suitable modelling techniques to these unique datasets that the full power of the twin model can be fully realised.

Chapter 3

3. Neurological Abnormalities in Twins with Schizophrenia

3.1. Abstract

3.1.1. While neurological abnormalities (NAs) are well recognised in schizophrenia, their aetiology, and specifically their genetic and environmental determinants are poorly understood. Thus their utility as endophenotypes is unknown.

3.1.2. 63 twin pairs, varying in their zygosity and concordance for schizophrenia, and 73 unaffected control twin pairs were examined for total, primary and integrative NAs using the Neurological Evaluation Scale.

3.1.3. NAs were increased in probands with schizophrenia compared to non-schizophrenic co-twins and to healthy control twins. There were no significant differences between patients, whether they were from concordant or discordant pairs. NAs in the non-psychotic co-twins from discordant pairs were increased compared to control twins. There were no significant differences in NAs between the non-schizophrenic co-twins from monozygotic (MZ) and dizygotic (DZ) discordant pairs, though the within pair correlations were greater in the MZ compared to DZ pairs. NAs were modified in all groups by pre-morbid schizotypal traits, and in patients by anti-psychotic medication.

3.1.4. Thus NAs in schizophrenia are determined in part by genetic risk for the illness and the presence of premorbid schizotypal traits. However anti-psychotic medication confers additional risk for NAs. NAs meet some criteria for being an endophenotype.

3.2. Introduction

3.2.1. Neurological abnormalities (NAs) are frequently noted in patients with schizophrenia (Kraepelin 1919; Buchanan and Heinrichs 1989; Keshavan, Sanders et al. 2003; Chan, Xu et al. 2010) but their pathophysiological significance remains elusive.

3.2.2. Family studies have consistently reported increased rates of NAs in the non-schizophrenic relatives of probands, including parents (Rossi, Decataldo et al. 1990; Kinney, Yurgelun Todd et al. 1991; Flyckt, Sydow et al. 1999), siblings (Ismail, Cantor-Graae et al. 1998; Egan, Hyde et al. 2001; Mechri, Bourdel et al. 2009; Mechri, Gassab et al. 2010), and offspring (Marcus, Hans et al. 1985; Schubert, Cantor-Graae et al. 2002; Schubert and McNeil 2004; Prasad, Sanders et al. 2009). NAs are highly correlated within families (Ismail, Cantor-Graae et al. 1998; Yazici, Demir et al. 2002) and there have been claims that they are determined by the degree of genetic loading for

schizophrenia within the family (Griffiths, Sigmundsson et al. 1998) and they are heritable and thus on the basis of this evidence that they could act as an endophenotype marker for the disorder (Hui, Wong et al. 2009).

3.2.3. Some studies of MZ twins discordant for schizophrenia (Mosher, Pollin et al. 1970; Cantor-Graae, McNeil et al. 1994; Niethammer, Weisbrod et al. 2000) have shown that the non-schizophrenic co-twins lie midway between probands and healthy controls in the extent of NAs detected.

3.2.4. Cantor-Graae (Cantor-Graae, McNeil et al. 1994) observed an association between obstetric complications and NAs in both the probands and their well co-twins, replicating this finding in a later study of non-twin relatives (Cantor-Graae, Ismail et al. 2000). They concluded that the NAs reflect the impact of environmental agents in genetically sensitised individuals, and additionally speculated that patients from MZ discordant pairs are subject to greater environmental effect than those from concordant pairs, who had a 'more genetic' form of the illness.

3.2.5. Griffith et al (Griffiths, Sigmundsson et al. 1998) divided NAs into primary and integrative subscales. Primary NAs were postulated to reflect dysfunction that can be detected at routine neurological examination, and include cranial nerve signs, eye movement abnormalities, lateralising limb pyramidal and frontal release signs. Integrative NAs were thought to reflect dysfunction in the integration of activity within the sensory and motor systems, and include dysdiadokokinesia, and the sequencing of complex motor acts, such as the fist-edge-palm test. The authors found that primary NAs were over represented in non-familial, or sporadic, cases of schizophrenia, and were not elevated in their well relatives. In contrast, integrative NAs were increased both in probands and unaffected relatives from multiply affected families. This suggested that primary NAs were a reflection of environmentally induced neurological damage in 'less familial and more environmental' patients, while integrative NAs were determined by genetic loading for schizophrenia and were found in the 'more familial' cases and their relatives.

3.2.6. Following this line of reasoning, I studied groups of twins varying in their zygosity and concordance for schizophrenia to test the following hypotheses:

3.2.6.1. Total NAs would be greater in twins with schizophrenia than control twins.

3.2.6.2. Twins with schizophrenia from concordant MZ pairs would have proportionally more integrative NAs, reflecting their greater genetic aetiological load, while those from discordant MZ pairs would have proportionally more primary NAs, reflecting their relatively greater environmental load.

3.2.6.3. Non schizophrenic co-twins from discordant pairs would have greater NAs, principally integrative, than control twins, but fewer than their ill co-twins. This would reflect their genetic, but lack of environmental, risk.

3.2.6.4. More NAs would be detected in non-schizophrenic co-twins from MZ discordant compared to DZ discordant pairs, reflecting their greater genetic proximity to their ill twin probands. This genetically determined effect would also be reflected in greater within pair similarities for NAs; MZ control>MZ discordant>DZ discordant.

3.3. Methods

3.3.1. Recruitment

3.3.1.1. One hundred and thirty six twin pairs contributed data to this component of the Twin Study after Multi Centre Research Ethics Committee approval had been granted.

3.3.1.2. Patients were referred by their treating psychiatrist. Control subjects were recruited from the Institute of Psychiatry Volunteer Twin Register and by advertisement in national media.

3.3.1.3. Exclusion criteria applied to all groups were a history of neurological illness, or of systemic illness with known neurological complication, a history of head injury associated with loss of consciousness of more than one minute, and current substance misuse or dependence.

3.3.1.4. All subjects gave written informed consent before participating.

3.3.2. Clinical Assessment

3.3.2.1. All subjects underwent the same extensive clinical assessment. Twin zygosity was determined by assessment of twelve highly polymorphic micro-satellite markers or standardised twin likeness questionnaire. Structured clinical interviews were performed by two trained psychiatrists using the Schedule for Affective Disorders and Schizophrenia-Lifetime version (Endicott and Spitzer 1978), augmented with further clinical information, from which DSM IV (American Psychiatric Association 1994) diagnoses were made. Current psychotic symptoms in the probands were assessed using the Scales for the Assessment of Positive and Negative Symptoms, SAPS (Andreasen 1983) and SANS (Andreasen 1983). Premorbid traits were assessed in a subset of subjects (62 pairs) by maternal interview using the Premorbid Assessment of Schizoid and Schizotypal Traits (PSST) (Foerster, Lewis et al. 1991). Handedness was determined using the Annett scale (Annett 1970). Data on medication history was collected at the time of assessment and side effects were rated using the Targeting Abnormal Kinetic Effects (TAKE) scale (Wojcik, Gelenberg et al. 1980).

Table 3.1.

Characteristics of the Twins

	MZ Concordant	MZ Discordant Ill	MZ Discordant Well	MZ Control	DZ Discordant Ill	DZ Discordant Well	DZ Control	Group Comparison F or X2 (df) p
Number of Subjects	60	21	21	110	12	12	36	
(Pairs)	(30)	(21)	(21)	(55)	(12)	(12)	(18)	
Age								
Mean (SD)	35.9 (10.2)	32.1 (11.9)	32.1 (12.0)	39.0 (12.1)	37.8 (12.7)	37.8 (12.6)	42.8 (12.2)	212 (7,136) <0.001
Gender % Female	29	41	41	49	46	46	52	3.55 (4) 0.48
Social Class Parents	2.60	2.64	2.64	2.67	2.31	2.31	2.67	1.91 (4) 0.75
Education Years								
Mean SD	13.3 (3.1)	11.8 (2.0)	12.3 (2.3)	13.8 (2.9)	14.0 (2.9)	15.5 (2.5)	13.4 (2.9)	627 (7,135) <0.001
Age at Diagnosis								
Mean SD	23.4 (7.0)	21.2 (6.0)	NA	NA	20.4 (4.5)	NA	NA	330 (3,133) <0.001
Handedness								
%Right	86	76	81	86	69	76	93	6.22 (6) 0.40
Other Axis I Diagnoses:								
Major Depression	NA	NA	11	13	NA	8	5	
Panic			4	1		3	1	
Generalised Anxiety			2	-		2	-	
Obsessive Compulsive			1	-		1	-	
Phobia			4	1		4	1	
SAPS								
Mean SD	7.7 (4.7)	6.6 (3.9)	NA	NA	5.6 (5.5)	NA	NA	58 (3,86) <0.001
SANS								
Mean SD	10.2 (5.0)	10.7 (6.8)	NA	NA	9.7 (7.6)	NA	NA	74 (3,86) <0.001
PSST								
Mean SD	10.6 (3.2)	11.3 (2.8)	10.7 (3.0)	8.1 (1.2)	10.9 (4.4)	8.1 (1.5)	8.0 (1.9)	4.1 (6,61) 0.016
Medication Cpz Equiv.								
Mean SD	612 (407)	602 (372)	NA	NA	561 (401)	NA	NA	50 (3,131) <0.001
Side Effects TAKE								
Mean SD	6.2 (3.4)	6.7 (4.4)	NA	NA	8.3 (4.8)	NA	NA	63 (3,88) <0.001

Legend Table 3.1

MZ, Monozygotic; DZ, Dizygotic; SAPS, Scale for the Assessment of Positive Symptoms; SANS, Scale for the Assessment of Negative Symptoms; PSST, Premorbid Assessment of Schizoid and Schizotypal Traits; Cpz Equiv, Chlorpromazine Equivalents; TAKE, Targeting Abnormal Kinetic Movements; NA, Not Applicable

3.3.3. Participants

3.3.3.1. The final study groups consisted of 30 pairs of MZ twins concordant for schizophrenia or schizoaffective disorder; 21 pairs of MZ twins and 12 pairs of DZ twins discordant for schizophrenia, in which the co-twin was free of any psychotic illness. Finally 55 pairs of MZ and 18 pairs of DZ control twins with no personal or family history to second degree relative, of a psychotic or schizophrenia spectrum disorder.

3.3.3.2. The probability that any of the discordant pairs would become concordant for schizophrenia in the future was low as an average of 10.24 (SD=8.82) years in the MZ, and 17.3 (SD=12.31) years in the DZ pairs, had elapsed since the onset of the illness in the probands (Belmaker, Pollin et al. 1974).

3.3.3.3. All subjects with schizophrenia were clinically stable at the time of assessment, with no recent changes to their medication.

3.3.3.4. Thirty nine of the non-schizophrenic co-twins from the discordant groups satisfied DSM IV criteria for a lifetime axis I disorder (Table 3.1). None were unwell at the time of testing nor taking any psychotropic medication. Within the MZ and DZ control groups 22 individuals satisfied DSM IV criteria for a lifetime axis I disorder, none were unwell at the time of testing and none were taking any psychotropic medication (Table 3.1).

3.3.4. Neurological Examination

3.3.4.1. The neurological examination was performed by one of two post Membership trained psychiatrists (MP and ND) blind to medication status, according to an expanded and previously validated version of the Neurological Evaluation Schedule (NES) (Buchanan and Heinrichs 1989; Griffiths, Sigmundsson et al. 1998) Table 3.2. This assessed thirty independent NAs; fifteen of the variables were assessed bilaterally giving a total of forty-five scored variables. This expanded version produced a Total score and scores on two subscales for Primary and Integrative NAs, hypothesised to reflect dysfunction in different CNS functional domains (Griffiths, Sigmundsson et al. 1998).

3.3.4.2. Primary NAs are thought to reflect localised cerebral pathology while integrative NAs to reflect dysfunction in the integration of activity within and between the sensory and motor systems.

3.3.4.3. The NES was administered in a standardised manner for each item and in a set order.

3.3.4.4. The three subscales from the original NES (Buchanan and Heinrichs 1989), (sensory integration, motor co-ordination and motor sequencing) were left unchanged (items scored on an anchored three point scale from 0=no abnormality to 2=marked impairment, except for the snout and suck reflexes which were scored as either 0 or 2). For the additional items, in the primary subscale, we used scoring from Griffiths et al (Griffiths, Sigmundsson et al. 1998), using a three point scale 0=no abnormality to 2=marked impairment according to the presence and severity of any abnormality. Conservative criteria for the determination of NAs were used. Scores for total, primary and integrative NA were calculated by producing the mean individual scores for the scales defined in Table 3.2.

3.3.4.5. Both psychiatrists were trained using a standardised schedule and video teaching tool. Inter-rater reliability was calculated by both physicians rating a sub-sample of twenty-six subjects randomly selected from the study group. Agreement rates were good for the two subscales: primary intra-class correlation coefficient (ICC)=0.79, integrative ICC=0.83.

Table 3.2.

Neurological Examination Schedule

Total Score

Primary Function	**Integrative Function**
Cranial nerve palsy (right and left)	Audio-visual integration
Smooth pursuit	Stereognosis (right and left)
Saccade to target	Graphaesthesia (right and left)
Saccade to command	Extinction
Synkinesis	Right/left confusion
Gaze impersistence	Tandem walk
Convergence	Rapid alternating movements (right and left)
Tone increase (right and left)	Finger thumb opposition test (right and left)
Limb hypereflexia (right and left)	Finger-nose test (right and left)
Babinski sign (right and left)	Fist-ring test (right and left)
Romberg sign	Fist-edge-palm test (right and left)
Chorea (right and left)	Oszeretski test
Tremor (right and left)	
Mirror movements (right and left)	
Glabellar reflex	
Snout reflex	
Grasp reflex (right and left)	
Suck reflex	

3.4. Data Analyses

3.4.1. The main objective of the statistical analysis was to compare values on the three dependent variables (i) total NAs, (ii) primary NAs and (iii) integrative NAs between the groups (MZ concordant ill, MZ discordant ill, DZ discordant ill, MZ discordant well, DZ discordant well, MZ and DZ control twin pairs).

3.4.2. The genetic relatedness of twin pairs and the likely within-pair correlations violate ANOVA's assumption of independence. In addition, the dependent variables in some groups suffered from floor effects (see Figures 3.1-3.3). Consequently group differences on NA variables were analysed using a regression model with standard errors that are robust against correlations within twin pairs and departures from normality (Binder 1983). The model uses dummy variables for group as independent variables. The robust sandwich estimator provides robust standard errors (and therefore robust confidence intervals and p-values) which give accurate assessments of the sample-to-sample variability of the parameter estimates even when the model is mis-specified, including when observations are non-independent, i.e. cluster-correlated as is the case in twins. Using robust regression the parameter estimate may be biased but the 95% confidence interval will be accurate, giving 95% confidence that the true parameter estimate lies within its range (Rogers 1993; Williams 2000).

3.4.3. Overall group comparisons followed by post-hoc pair-wise comparisons were carried out. The choice of group comparisons was guided by the hypotheses (i)-(iv) listed in the introduction, thus we considered the following comparisons:

- MZ Concordant Ill, MZ Discordant Ill, DZ Discordant Ill vs MZ & DZ Control
- MZ Concordant Ill vs MZ Discordant Ill
- MZ Discordant Ill vs MZ Discordant Well
- DZ Discordant Ill vs DZ Discordant Well
- MZ Discordant Well vs MZ Control
- DZ Discordant Well vs DZ Control
- MZ Discordant Well vs DZ Discordant Well

3.4.4. To control for multiple testing, pairwise comparisons were carried out only when a significant overall difference was detected and the Bonferroni correction used to judge statistical significance throughout.

3.4.5. Regression and logistic regression with robust standard errors were used to compare demographic and clinical variables between groups while taking account of twin clusters. Age, gender, social class of parents at subjects' birth, and educational achievement were considered potential confounders of the group effects on NAs.

Whenever one of the terms contributed significantly to the prediction of the NA variable, it was included in the model to adjust group effects for confounder differences.

3.4.6. Within the patient groups, the effects of patients' age at onset, medication and psychotic symptoms were evaluated by adding the variables to the model for the NA outcomes after including group effects. To determine if schizotypal personality trait score affected NA either as an interaction with group or as a main effect, these terms were added to the model.

3.4.7. Intraclass correlation coefficients (ICC) were calculated within the MZ control, MZ discordant and DZ discordant groups to further test hypothesis iv. The ICCs measure the degree of (positive) correlation between the scores of the members of each twin pair. A bootstrapping approach was used to compare the ICC in the MZ discordant group with each of the other two groups. The bootstrap algorithm allowed for effects of predictor variables on NAs and resampled twin pairs within each group to generate percentile confidence intervals for ICCs and tests for zero differences.

3.4.8. All analyses were carried out in Stata 8 (Stata Corporation, 2003).

3.5. Results

3.5.1. Power Calculation

3.5.1.1. Previous work (Cantor-Graae, McNeil et al. 1994) found a difference in mean NA score between schizophrenic and control groups of 3.7 and a within group standard deviation of 2.5. On this basis, I calculated that at least 11 unrelated subjects in each group would be needed to have 90% power to detect such an effect using a two sided independent samples t-test at the 5% significance level.

3.5.2. Group Demographics

3.5.2.1. The groups differed significantly in age and years spent in full time education (Table 3.1). Probands from both of the discordant groups were younger ($p<0.001$) at initial diagnosis than the MZ concordant pairs; they had statistically fewer positive ($p<0.001$), and in the case of the DZ pairs, fewer negative symptoms, and were on lower doses of antipsychotic medication, expressed in chlorpromazine equivalents ($p<0.001$). The DZ discordant probands experienced more side effects (TAKE) ($p<0.001$).

3.5.2.2. Age ($p<0.001$) and educational achievement ($p=0.002$) significantly predicted NA scores, while gender ($p=0.79$), and parental social class ($p=0.45$) did not.

3.5.2.3. The group comparisons for the NA scores were therefore adjusted for age and educational achievement to control for potential confounding effects.

3.5.3. Total Neurological Abnormalities

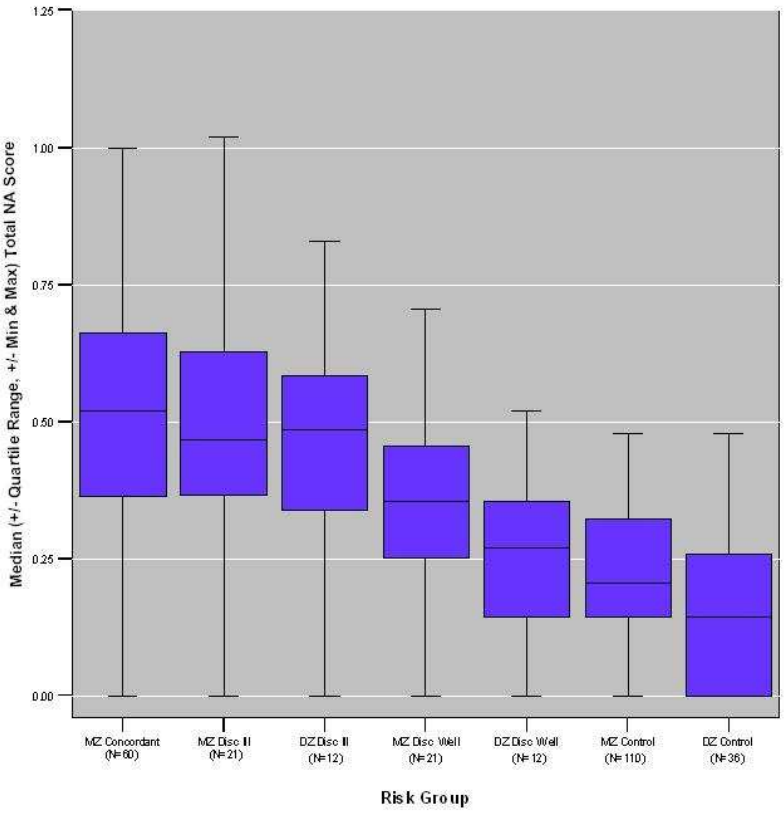


Figure 3.1.

Box plots demonstrating distribution of Total NA score within groups.

Disc, Discordant; N, Number of subjects; Min, Minimum; Max, Maximum; NA, Neurological Abnormality.

Reprinted from: Picchioni MM, Toulopoulou T, Landau S, Davies N, Ribchester N, Murray R.M. (2005)Neurological abnormalities in twins. Biological Psychiatry 59, 4 341-348.

3.5.3.1. The observed total NA scores are illustrated in Figure 3.1 and the results of the formal group comparisons in Table 3.3.

3.5.3.2. An overall effect of group on total NA score was detected ($p<0.0001$).

3.5.3.3. In accord with hypothesis i, patients with schizophrenia (all those from MZ concordant, MZ discordant and DZ discordant pairs) had more total NAs than control twins ($p<0.001$), while the MZ concordant and MZ discordant schizophrenic patients did not differ significantly from each other ($p=0.82$).

		Group Comparison						
		Schizophrenic proband vs Control	MZ Conc vs MZ Disc Ill	MZ Disc Ill vs MZ Disc Well	DZ Disc Ill vs DZ Disc Well	MZ Disc Well vs MZ Control	DZ Disc Well vs DZ Control	MZ Disc Well vs DZ Disc Well
Total	Overall Group Comparison	F(7, 132)=81.1, p<0.0001						
	Pairwise Test df=132	t=9.65 p<0.001**	t=-0.16 p=0.88	t=3.97 p<0.001**	t=2.24 p=0.027*	t=3.02 p=0.003**	t=1.89 p=0.061	t=1.08 p=0.28
	Group Difference (95% CI)	0.32 0.25 to 0.38	-0.015 -0.14 to 0.11	0.16 0.07 to 0.24	0.20 0.023 to 0.37	0.14 0.04 to 0.23	0.12 -0.01 to 0.25	0.079 -0.066 to 0.22
Primary	Overall Group Comparison	F(7, 132)=42.8, p<0.0001						
	Pairwise Test df=132	t=8.31 p<0.001**	t=0.33 p=0.74	t=1.99 p=0.048*	t=2.76 p=0.007**	t=2.58 p=0.011*	t=2.11 p=0.037*	t=1.15 p=0.25
	Group Difference (95% CI)	0.29 0.22 to 0.36	0.028 -0.14 to 0.19	0.10 0.0006 to 0.21	0.20 0.05 to 0.34	0.144 0.034 to 0.26	0.13 0.007 to 0.25	0.086 -0.06 to 0.23
Integration	Overall Group Comparison	F(7, 132)=67.6, p<0.0001						
	Pairwise Test df=132	t=7.90 p<0.001**	t=0.07 p=0.94	t=3.79 p<0.001**	t=1.48 p=0.14	t=1.89 p=0.061	t=1.76 p=0.08	t=0.34 p=0.73
	Group Difference (95% CI)	0.37 0.28 to 0.46	0.006 -0.13 to 0.14	0.24 0.12 to 0.37	0.19 -0.06 to 0.45	0.11 -0005 to 0.23	0.16 -0.019 to 0.34	0.033 -0.16 to 0.23

Table 3.3.

Neurological Abnormalities (NA) Test Results

Legend Table 3.3

** Significant at $p<0.007$ corrected for multiple comparisons, * Significant at $p<0.05$ uncorrected, all tests were adjusted for subject age and educational achievement. MZ, Monozygotic; DZ, Dizygotic; Conc, Concordant; Disc, Discordant

3.5.3.4. Probands with schizophrenia from MZ discordant and DZ discordant pairs had more total NAs than their non-schizophrenic co-twins, ($p<0.001$ and $p=0.027$ respectively), supporting hypothesis iii, but the DZ pair differences were not statistically significant after Bonferroni correction.

3.5.3.5. The non-schizophrenic co-twins in discordant MZ pairs had more total NAs than control twins, ($p=0.003$), again supporting hypothesis iii, and while there was a similar trend in the DZ non-schizophrenic co-twins, it did not reach statistical significance ($p=0.061$).

3.5.3.6. Contrary to hypothesis iv, the non-schizophrenic co-twins from MZ and DZ discordant pairs did not differ significantly in total NA score ($p=0.28$).

3.5.4. Primary Neurological Abnormalities

3.5.4.1. The observed Primary NA scores are illustrated in Figure 3.2 and the formal group comparisons are shown in Table 3.3.

3.5.4.2. An overall effect of group on primary NA score was detected ($p<0.0001$).

3.5.4.3. Patients with schizophrenia from MZ concordant, MZ discordant and DZ discordant pairs had more primary NA than control twins ($p<0.001$) but, contrary to hypothesis ii, probands from MZ discordant pairs did not have significantly more primary NA than those from MZ concordant pairs ($p=0.79$).

3.5.4.4. As predicted in hypothesis iii, probands with schizophrenia from MZ discordant and DZ discordant pairs had more primary NA than their non-schizophrenic co-twins ($p=0.048$, $p=0.007$ respectively) though only the DZ pair differences remained statistically significant after Bonferroni correction.

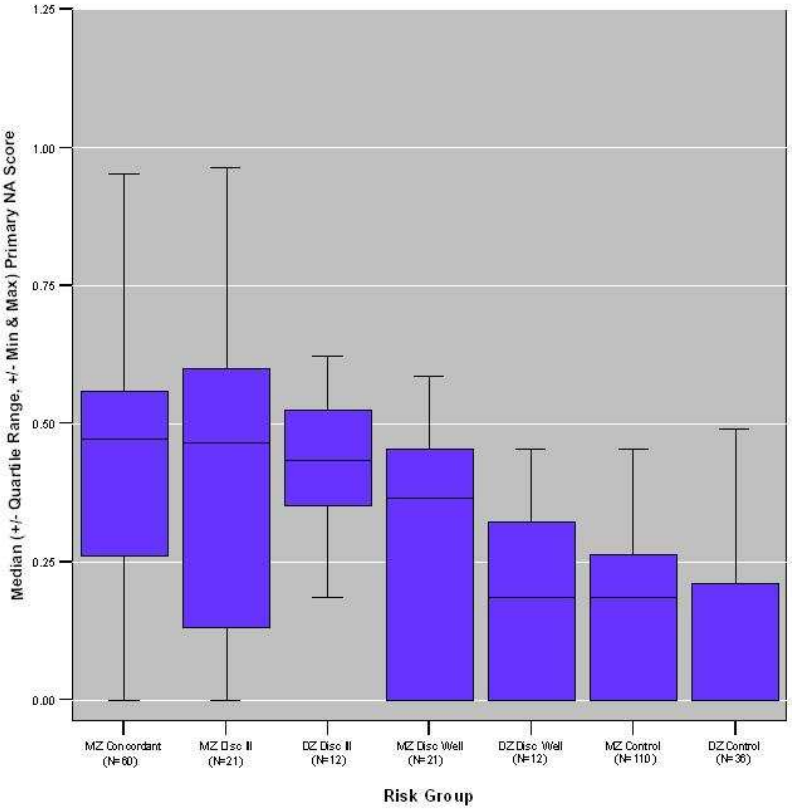


Figure 3.2.

Box plots demonstrating distribution of Primary NA score within groups.

Disc, Discordant; N, Number of subjects; Min, Minimum; Max, Maximum; NA, Neurological Abnormality.

Reprinted from: Picchioni MM, Toulopoulou T, Landau S, Davies N, Ribchester N, Murray R.M. (2005)Neurological abnormalities in twins. Biological Psychiatry 59, 4 341-348.

3.5.4.5. Contrary to hypothesis iii, there were trends for the non-schizophrenic co-twins of both the MZ and DZ discordant pairs to have more primary NA than control twins (p=0.011 and p=0.037 respectively), though neither were significant after Bonferroni correction.

3.5.4.6. The non-schizophrenic co-twins from MZ and DZ discordant pairs again did not differ significantly from each other on primary NA scores (p=0.25).

3.5.5. Integrative Abnormalities

3.5.5.1. The observed integrative NA scores are illustrated in Figure 3.3 and the formal group comparisons are shown in Table 3.3.

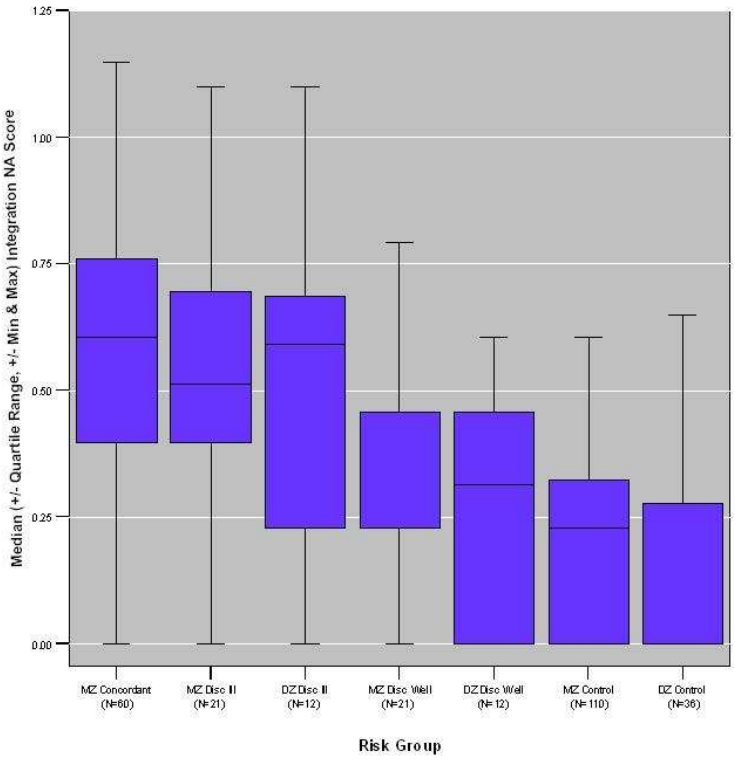


Figure 3.3.

Box plots demonstrating distribution of Integration NA score within groups.

Disc, Discordant; N, Number of subjects; Min, Minimum; Max, Maximum; NA, Neurological Abnormality.

Reprinted from: Picchioni MM, Toulopoulou T, Landau S, Davies N, Ribchester N, Murray R.M. (2005)Neurological abnormalities in twins. Biological Psychiatry 59, 4 341-348.

3.5.5.2. An overall effect of group on integrative NA score was detected ($p<0.0001$).

3.5.5.3. In accord with hypothesis i, patients with schizophrenia from MZ concordant, MZ discordant and DZ discordant pairs had more integrative NA than control twins ($p<0.001$), while, contrary to hypothesis ii, patients from MZ concordant and MZ discordant pairs did not differ significantly from each other ($p=0.91$).

3.5.5.4. Contrary to hypothesis iii, probands from the MZ discordant group had more integrative NA than their non-schizophrenic co-twins ($p<0.001$).

3.5.5.5. Probands from DZ discordant pairs did not have significantly more integrative NA than their non-schizophrenic co-twins (p=0.14).

3.5.5.6. We did not find a significant difference between the non-schizophrenic co-twins from either the MZ or DZ discordant groups and control twins (p=0.061 and p=0.080) for integrative NA.

3.5.5.7. Finally, and contrary to hypothesis iv, there was no significant difference between the non-schizophrenic co-twins from MZ and DZ discordant pairs for integrative abnormality scores (p=0.73).

3.5.6. Intraclass Correlations

3.5.6.1. The ICCs adjusted for age and educational achievement for the MZ control, MZ discordant and DZ discordant groups are shown in Table 3.4.

Table 3.4.

Intra-class correlations within twin pairs

	Total Abnormalities		Primary Abnormalities		Integration Abnormalities	
	ICC	95% CI*	ICC	95% CI*	ICC	95% CI*
MZ Control (n=55++)	65.7%	62.7 to 83.3%	53.4%	48.6 to 78.1%	48.6%	44.2 to 77.6%
MZ Discordant (n=21++)	52.3%	31.8 to 92.6%	49.6%	31.5 to 86.2%	16.4%	0 to 89.9%
DZ Discordant (n=12++)	0%	0 to 64.7%	0%	0 to 65.5%	0.0%	0 to 65.0%
Pairwise group comparisons						
Comparison	ICC difference	P-value +	ICC difference	P-value +	ICC difference	P-value +
MZ Control – MZ Discordant	13.4%	0.51	3.8%	0.82	32.2%	0.38
MZ Discordant – DZ Discordant	52.3%	0.025	49.6%	0.038	16.4%	0.74

Legend Table 3.4

* Bootstrap percentile confidence interval CI based on 1999 bootstrap simulations

+ Bootstrap test of constant intra-class correlation ICC in the two groups based on 1999 bootstrap simulations

++ Number of pairs

3.5.6.2. The distribution of the ICC data was consistent with our a priori hypothesis iv.

3.5.6.3. The ICCs for the MZ discordant pairs were significantly greater than the DZ discordant pairs for both total (p=0.025) and primary (p=0.038) NAs.

3.5.6.4. However the ICCs for the MZ discordant twins did not differ significantly from the MZ control twins for any of the three NA scales.

3.5.7. Antipsychotic Medication Effects

3.5.7.1. Within the three patient groups, there was a significant effect of medication on total, primary and integrative NA score (t(128)=2.73, t(128)=2.85, t(128)=2.15 and p=0.007, p=0.005 and p=0.033, respectively).

3.5.7.2. This effect was mirrored for side effect ratings on total, primary and integrative NAs, (t(88)=4.01, t(88)=4.19and t(88)=2.66 and p<0.0001, p<0.001 and p=0.009 respectively).

3.5.7.3. These effects reflected greater medication doses and side effect scores being associated with increased NA scores.

3.5.8. Psychotic State and Trait Effects

3.5.8.1. Current psychotic symptoms did not have a significant effect on any of the three NA scores within patients from the three groups MZ concordant and MZ and DZ discordant, either as an interaction or main effect.

3.5.8.2. However there was a trend towards increased negative symptoms being associated with increased total and primary NA (t(56)=1.80, t(56)=1.89, and p=0.078, p=0.064, respectively).

3.5.8.3. Within the DZ discordant well group only 3 subjects scored non-zero on PSST rating, so I excluded this group from regression fitting and subsequent analysis of PSST effects.

3.5.8.4. There was no significant interaction between PSST score and group on any of the three NA scores. However there was a main effect of PSST on both total (t(61)=54, p=0.039), and primary (t(61)=50, p=0.030) NA scores. Thus, increasingly abnormal personality was associated with increasing NA score.

3.6. Conclusions

3.6.1. General

3.6.1.1. The main findings are that NAs are, as predicted, increased both in probands with schizophrenia probands (hypothesis i) and their non-schizophrenic co-twins (hypothesis iii) compared to controls.

3.6.1.2. I confirmed that the non-schizophrenic co-twins occupy a position intermediate between their ill co-twins and healthy twins, but was unable to show from the comparison of means analysis, that the genetic proximity of the non-schizophrenic co-twins from discordant pairs to their respective probands determined their level of NA, i.e. MZ>DZ (hypothesis iv). However, the ICC data from these pairs give indirect support to this hypothesis.

3.6.1.3. NAs of probands did not vary with their concordance for schizophrenia (contrary to hypothesis ii) nor psychotic symptomatology, but were influenced by antipsychotic medication.

3.6.1.4. An effect was also detected across all groups between schizotypal personality traits and NAs. Chapter 4 will show that this further implicates a genetic role in their aetiology.

3.6.1.5. Finally, the results do not support the division of NA into primary and integrative scores on aetiological grounds.

3.6.1.6. These findings implicate familial, and more specifically genetic factors, in determining the level of NA detected in the well co-twins of patients with schizophrenia. However non-shared environmental, including illness specific factors must have had an additional effect as probands, particularly those from MZ discordant pairs, expressed significantly more NA than their genetically identical, but well, co-twins. Antipsychotic medication played a part in this final effect.

3.6.2. Non-Schizophrenic Co-twins

3.6.2.1. Non-schizophrenic co-twins from MZ and DZ discordant pairs manifested elevated levels of NA compared to controls, suggesting that NAs reflect a familial factor related to schizophrenia. The ICC analysis, showing greater similarity within MZ than DZ discordant pairs, clarify that NA are determined in part by the co-twins' genetic proximity to the proband, and consequently their genetic risk for the disorder. However, contrary to our hypothesis iii, we found significantly increased total and primary, rather than integrative, NA in the non-schizophrenic co-twins from the discordant pairs, suggesting that the division of NA into Primary and Integrative scales does not accurately reflect genetic risk as Griffith et al (1998) originally proposed.

3.6.2.2. Cantor-Graae et al (Cantor-Graae, McNeil et al. 1994) speculated that MZ discordant compared to MZ concordant pairs, might represent a peculiarly environmental variant of the illness and consequently that the well co-twins of discordant pairs may have inherited relatively little genetic risk for schizophrenia. Evidence from a variety of other sources, epidemiological (Cardno, Marshall et al. 1999; Sullivan, Kendler et al. 2003), neuro-cognitive (Cannon, Huttunen et al. 2000),

neuroimaging, (Cannon, Thompson et al. 2002; van Erp, Saleh et al. 2004; van Haren, Picchioni et al. 2004), together with data from this study, begins to suggest that on balance this is unlikely to the case. Non-schizophrenic co-twins from MZ and DZ discordant pairs consistently manifest deficits that place them in a position intermediate between controls and those with clinical schizophrenia.

3.6.2.3. While the co-twins from MZ and DZ discordant pairs did not differ significantly in NA, the ICCs for MZ discordant pairs was more similar for Total and Primary NA than for DZ pairs. This suggests that Total and Primary, rather than Integrative NA, are influenced by genetic factors, and more specifically that their expression in well relatives reflects their genetic risk for the illness. This finding for Primary NA was not that hypothesised and could reflect the lack of specificity of the NA examination, coupled with aetiological and pathophysiological heterogeneity within the sample.

3.6.2.4. The failure of the comparison of means approach to detect significant differences between the non-schizophrenic co-twins from MZ and DZ discordant pairs could reflect the limited power of the study to detect differences between two non-schizophrenic groups, each of limited size, defined by their degree of genetic risk. Alternatively the NA may in fact be determined by shared environmental factors, and my failure to detect differences between the two groups would then be a genuine finding; however that would inconsistent with the ICC data if the equal environments assumption in twins is true.

3.6.2.5. There have been no published studies that examined NAs in MZ and DZ discordant twins. However, previous neuro-imaging studies in twins (Cannon, Thompson et al. 2002) and multiply effected families (McDonald, Bullmore et al. 2004), have used theoretical genetic loading scales to model the influence of the genetic risk for schizophrenia on cerebral volume. Results indicated that relatives demonstrate a similar distribution of deficits to probands, and that the magnitude of those deficits is determined at least in part by their degree of genetic risk; this could reflect the greater sensitivity for what ever reason, of structural MRI measures compared to NAs, to index the genetic risk of schizophrenia.

3.6.3. Probands

3.6.3.1. My findings imply that NAs are a robust finding in schizophrenia, determined in part by the inherited genetic risk for the disorder. However since MZ twins with schizophrenia express greater levels of NA, across all three scales, than their co-twins they must also be influenced by non-genetic factors. These are not related to positive

psychotic symptoms or other measures of illness severity (state), but the data do support the logical conclusion that NAs are increased by antipsychotic medication.

3.6.3.2. Across the MZ patient groups (MZ concordant and MZ discordant ill), the distribution of total, primary and integrative NA abnormalities did not differ significantly. While the MZ discordant twins are unlikely to represent pairs of very low genetic load, a structural MRI study by our group (van Haren, Picchioni et al. 2004) has suggested that probands from discordant pairs may be more sensitive to environmental aetiological events, expressed as larger lateral ventricular volumes, or that the concordant twins have experienced a greater genetic load (Ettinger, Picchioni et al. 2007). This we concluded is more likely to reflect a more subtle shift in the gene-environment interaction in some discordant pairs than a more absolute model. The similar distribution of NA scores across the patient groups leads to the conclusion that while NAs do reflect this interaction between genetic and unshared environmental risk factors for schizophrenia, the pattern of expression is largely insensitive to the balance between the two.

3.6.3.3. Within the patients in this specific sample, those from discordant pairs were younger at original diagnosis, but otherwise less symptomatic and on lower doses of medication. These clinical differences did not result in significant between group differences on the NA scores. Studies contrasting concordant and discordant MZ pairs on measures of clinical severity have produced mixed results.

3.6.4. Antipsychotic Medication Effects

3.6.4.1. The association between NAs and antipsychotic medication is plausible given their pharmacological properties and evidence that they cause brain changes (Dazzan, Morgan et al. 2004; Lieberman, Tollefson et al. 2005). While some studies have shown a link, as a statistical relationship, between NAs and antipsychotic medication (Gupta, Andreasen et al. 1995), the majority have failed to detect this.

3.6.4.2. In this study, the effect in the probands was related both to current antipsychotic dose, and measured extra-pyramidal side effects. While scales for NAs and extra-pyramidal side effects may be measuring the same phenomena, I tried to guard against this by selecting the TAKE scale to directly assess extra-pyramidal side effects, this scale being less prone to contamination by NA variables than other side effect rating scales.

3.6.4.3. It was not possible to covary for the effects of medication in the analysis model as neither the well co-twins nor controls were on any medication. One hypothesis might be that rather than being a direct consequence of antipsychotic medication, NAs may act as a marker of subjects who are more prone to experience extra-pyramidal side

effects to antipsychotics. Support for this comes from observations of 'extra-pyramidal side effects' in treatment naïve patient groups (Gupta, Rajaprabhakaran et al. 1995; Wolff and O'Driscoll 1999; Shibre, Kebede et al. 2002) and in those who later go on to develop symptoms (Leask, Done et al. 2002).

3.6.5. Schizotypy

3.6.5.1. NAs were associated with increased childhood schizotypal personality traits. The latter characteristics occur more commonly in those who later develop schizophrenia and correlate with familial risk for the disorder (Mata, Gilvarry et al. 2003).

3.6.5.2. In a recent study of neurocognitive deficits and schizotypal symptoms in schizophrenic twins, Johnson et al (Johnson, Tuulio-Henriksson et al. 2003) concluded that the presence of schizotypal symptoms alone did not confer risk for cognitive deficits without the additional presence of genetic risk for the illness. I have however shown in Chapter 4 of this thesis, that schizotypal traits correlate both genotypically and phenotypically with schizophrenia, that is that they share common risk genes. This finding is in agreement with Johnson's suggestion that susceptibility loci for schizophrenia manifest themselves both as neurocognitive deficits and schizotypal symptoms, while my data support the conclusion that their effects also extend to NA expression.

3.6.6. NAs as an Endophenotype

3.6.6.1. Against the endopheotype criteria outlined in Chapter 1, I have confirmed in this study that NA's are associated with schizophrenia. They are unrelated to the psychotic symptoms of the disorder, thus are state independent, but appear to be influenced by, or at least related to, its treatment with antipsychotic medication. NA's are found at higher rates in the families of patients than the general population, with some evidence that they co-segregate in those families. When assessed using a standardised scale by trained clinicians, I have shown that they can be reliably quantified. Unfortunately I was not able to assess their heritability as the sample was too small for genetic modelling, though the distribution of deficits suggested that they were influenced by genetic effects. Finally this study was unable to assess their 'genetic architecture'

3.7. Strengths and Weakness

3.7.1. Firstly, this study was hampered by a lack of blindness to diagnostic status. In preliminary assessments it was impossible to maintain blindness in the context of a clinical examination and so this was dropped as a feature of the study design.

3.7.2. Secondly, NAs are by definition subjective and prone to rater error. I guarded against this by thoroughly training two raters, using a well validated structured rating instrument, and monitored inter-rater reliability.

3.7.3. Thirdly, the subjects were not drawn from an epidemiological sample and consequently may have been prone to selection bias. This may have been compounded by the twin nature of this sample, since twins are more prone to obstetric complications, which can cause cerebral pathology manifesting as NAs (Pinborg, Loft et al. 2004).

3.7.4. Both our control sample and well co-twins from discordant pairs, contained subjects with other lifetime axis I disorders. In order to maintain diagnostic consistency between all the non-schizophrenic subjects (MZ and DZ discordant co-twins, and MZ and DZ controls), we applied the same psychiatric exclusion criteria to all. Consequently these groups contained subjects who fulfilled criteria for other lifetime axis I disorders. None of these 'well' subjects was symptomatic at the time of testing or taking any psychotropic medication. We considered that to introduce additional exclusion criteria for the controls, specifically that any past illness be an exclusion criterion, would inflate differences with the controls by making the latter 'super-well' in comparison to the discordant well co-twins. I repeated the analyses excluding all control subjects with a past axis I disorder. As predicted this tended to increase group differences, particularly between DZ discordant co-twins and the controls.

3.7.5. I made multiple statistical comparisons but only after a significant effect had been detected at the group level and post hoc results are quoted using the Bonferroni correction.

3.7.6. The failure of the study to detect significant differences between the well co-twins of discordant MZ and DZ pairs may well represent a type II error. This was the first study to directly compare MZ and DZ discordant groups on NA, the co-twins from these two groups differ only in their genetic proximity to the patient, and we can speculate consequently that the effect sizes will be small.

3.7.7. Strengths of this study include the fact that this sample represents the largest examination of NA in twins with schizophrenia, and the only one to include both MZ and DZ discordant pairs, as well as compare patients from MZ concordant and discordant pairs, to assess their suitability as endophenotype markers for schizophrenia. Rigorous structured diagnostic and assessment criteria were applied throughout and improved

statistical methods utilised to capitalise on the strengths of the twin model while avoiding issues of independence.

Chapter 4

4. Childhood Social Development and Personality in Twins and Siblings with Schizophrenia.

4.1. Abstract

4.1.1. Abnormalities in early social development and personality are present in patients with schizophrenia and their unaffected relatives. In this study I aimed to establish both the degree to which these childhood and adolescent developmental abnormalities are genetically determined, and to what extent they share genetic risk with schizophrenia.

4.1.2. I used a combined twin and family cohort (n=531) to assess childhood and adolescent social adjustment and schizotypal personality traits in 98 twin pairs (n=196) varying in their zygosity and concordance for schizophrenia and 156 sibling clusters (n=335) varying in their concordance for schizophrenia.

4.1.3. Schizophrenia was significantly associated with childhood and adolescent deficits in social adjustment and schizotypal personality traits in adolescence. Additive genetic effects were the main source of these phenotypic correlations.

4.1.4. Thus abnormalities of social adjustment and personality are present in children and adolescents who will later develop schizophrenia. These deficits share common genetic risk with schizophrenia. They satisfy many endophenotype criteria for schizophrenia.

4.2. Introduction

4.2.1. The neurodevelopmental model (Weinberger 1987; Murray, O'Callaghan et al. 1992) suggests that the origins of the illness lie in early life and that patients manifest signs of abnormal neural function well before overt psychotic symptoms. This theory is based in part, on the observation of premorbid abnormalities in a wide variety of domains including schizotypal personality and social competency (Gupta, Rajaprabhakaran et al. 1995; Bailer, Brauer et al. 1996; Davidson, Reichenberg et al. 1999; Vourdas, Pipe et al. 2003; Addington and Addington 2005; Johnstone, Ebmeier et al. 2005). These deficits predict those who go on to develop schizophrenia (Johnstone, Ebmeier et al. 2005), acting as markers of future illness severity (Gupta, Rajaprabhakaran et al. 1995; Vourdas, Pipe et al. 2003; Addington and Addington 2005) and prognosis (Bailer, Brauer et al. 1996; Addington and Addington 2005). It is possible that these deficits could represent early facets of the illness, or alternatively risk factors for it.

4.2.2. Similar abnormalities accumulate in patients' non-psychotic relatives (Cannon-Spoor, Potkin et al. 1982; Foerster, Lewis et al. 1991; Maxwell 1992; Walshe, Taylor et al. 2007; Shapiro, Marcenco et al. 2009), suggesting but not proving that they are genetic in origin. This question though remains unanswered, since the familial aggregation could result from shared familial environmental factors; by virtue of their design the earlier studies could not discriminate between these two competing influences.

4.2.3. Combined twin and family studies utilising genetic modelling offer the optimal study design both to explore and quantify the extent to which genetic and environmental factors influence variation in, and covariation between traits. Developmental data in a schizophrenia twin and family sample can both establish the genetic and environmental influences on such traits, and quantify the genetic relationship between these facets and schizophrenia. A combined twin and family study design offers the additional advantages of increasing experimental sample size, reducing sample variance and offering a better approximation of the true population than a twin sample alone, while also increasing the study's power to discriminate between additive and dominant genetic effects. Sibling pairs can model for dizygotic (DZ) twin pairs on the basis that they share the same degree of genetic similarity.

4.2.4. I hypothesised:

4.2.4.1. firstly that childhood and adolescent social adjustment and personality will be more abnormal in patients with schizophrenia compared both to their non-psychotic relatives and healthy controls

4.2.4.2. secondly that their non-psychotic relatives will also be more abnormal than controls.

4.2.4.3. thirdly that genetic model fitting would show that shared genetic factors underlie both abnormal early social development and personality, and schizophrenia.

4.3. Methods

4.3.1. Recruitment

4.3.1.1. The subjects for this study participated in the Maudsley Twin (Picchioni, Toulopoulou et al. 2006; Toulopoulou, Picchioni et al. 2007) and Family (McDonald, Marshall et al. 2006) Studies of Schizophrenia.

4.3.1.2. A total of 531 individuals contributed to this study after Research Ethics Committee approval had been granted.

4.3.1.3. Recruitment was as described in 3.3.1.2. Twin and singleton probands with schizophrenia, and their relatives, were recruited nationally through referral by treating National Health Service (NHS) psychiatrists and voluntary support groups. Control twins and families were recruited nationally.

4.3.1.4. Exclusion criteria were as in 3.3.1.3 with in addition lack of a suitable parent informant.

4.3.1.5. All probands gave written informed consent for their parents to contribute after a detailed description of the study aims and method.

4.3.2. Clinical Assessment

4.3.2.1. All subjects directly assessed used the same methodology as 3.3.2.1. For sibling relatives not directly assessed, information regarding clinical status was obtained from the most reliable informant using the Family Interview for Genetic Studies (Maxwell 1992) with additional information from medical notes where available. Twin zygosity was determined from 12 highly polymorphic microsatellite markers (95%) or a twin likeness questionnaire (5%).

4.3.2.2. Developmental Assessment

4.3.2.2.1. Early social function and adjustment were assessed by parental interview (40%) and parental self-report questionnaire (60%) using a modified version of the Premorbid Social Adjustment scale PSA(Cannon-Spoor, Potkin et al. 1982; Foerster, Lewis et al. 1991). This assesses premorbid function on an eight point scale (0-7) of increasing deviance, over two developmental phases, childhood to the age of 11, and adolescence from 12 to 16 years. It assesses function in four developmental areas, social interactions, peer relationships, school performance and adaptation, and general interests, as well as psychosexual function in the adolescent period only. It produces a sum score for each developmental age phase.

4.3.2.2.2. Premorbid schizotypal personality traits were assessed using the Premorbid Assessment of Schizoid and Schizotypal Traits (PSST)(Foerster, Lewis et al. 1991). This is a seven item questionnaire, on which each item is rated on a four point scale (0-3) of increasing deviance using explicit anchor points, to produce a sum score. The items cover sociability, affect, suspiciousness and sensitivity, beliefs and behaviour, speech and isolated or peer group anti-social behaviour.

4.3.3. Participants

4.3.3.1. The final study group consisted of ninety-eight twin pairs (21 monozygotic (MZ) concordant for schizophrenia, 18 MZ discordant for schizophrenia in which the co-twin was free of any psychotic illness, 10 DZ discordant for schizophrenia and 29 MZ and 20 DZ healthy control twin pairs (with no personal or family history of a psychotic or schizophrenia spectrum disorder), and one hundred and fifty six sibling family clusters(Walshe, Taylor et al. 2007) (128 schizophrenia patients, 21 of their concordant ill siblings, 122 of their discordant (i.e. free of any psychotic illness)

siblings, and 85 healthy control siblings from unaffected families, the largest family contained eight siblings.

4.3.3.2. The probability that any of the discordant twin pairs would become concordant for schizophrenia was low as an average of 8.9 (SD=5.9) years in the MZ, and 16.5 (SD=11.2) years in the DZ pairs, had elapsed since the onset of the probands' illness (Belmaker, Pollin et al. 1974). 16 of the non-schizophrenic co-twins from discordant pairs, 13 of the unaffected siblings and 21 of the healthy controls met DSM-IV criteria for a lifetime axis I disorder, mainly major depressive disorder, though none were unwell at the time of testing.

4.3.4. Regression Analysis

4.3.4.1. The PSA and PSST scales generated three dependent variables, total childhood social adjustment (CSA), total adolescent social adjustment (ASA) and total premorbid schizotypal personality traits (PSST).

4.3.4.2. The main objective of this part of the statistical analysis was to compare values on these three dependent variables between the groups; MZ concordant ill, MZ discordant ill, MZ discordant well, siblings concordant ill, DZ twins & siblings discordant ill, DZ twins & siblings discordant well, with all the healthy control subjects.

4.3.4.3. As before the genetic relatedness of twin and siblings and the likely within-family correlations violate ANOVA's assumption of independence. Furthermore, there were outlying points in the PSA distributions, while the PSST variables in some groups suffered from floor effects. Consequently, group differences were analysed using a regression model with standard errors that is robust against correlations within families, departures from normality, and heterogeneity of covariance matrices (Binder 1983). The model uses dummy variables for group as independent variables.

4.3.4.4. I followed a similar analytical approach as in 3.4 and carried out overall group comparisons followed by post-hoc pair-wise comparisons. To control for multiple testing, pairwise comparisons were carried out only when there was a significant overall difference and Bonferroni correction used to judge statistical significance throughout.

4.3.4.5. Regression and logistic regression with robust standard errors were used to compare demographic and clinical variables between groups while taking account of family clusters. Age and gender were considered potential confounders of the later group effects on the dependant variables. All analyses were carried out in Stata 10 (StataCorp LP, Texas, USA, 2008).

Table 4.1

Demographics of twins & siblings

	MZ Cc ill N= 42	***Siblings Cc ill N= 21***	***MZ Dc ill N= 18***	***DZ Dc ill & Siblings Dc ill N= 117***	***MZ Dc well N=18***	***DZ Dc well & Siblings Dc well N=132***	***All Controls N=183***	***Group Comparison F or $\chi 2$ (df) p***
Age Mean *(SD)*	33.92 (8.88)	30.78 (5.52)	30.01 (9.23)	32.57 (7.62)	30.01 (9.23)	34.44 (8.15)	35.26 (10.62)	424 (11, 253) <0.001
Sex % Female	19	19	39	30	39	55	59	37.1 (8) <0.001
Years Education Mean *(SD)*	14.07 (3.16)	13.28 (3.53)	13.67 (3.07)	13.09 (2.45)	13.53 (2.65)	14.19 (2.94)	13.85 (10.76)	540 (11, 242) <0.001
Social Class Mean (SD)	2.29 (0.84)	2.33 (1.28)	2.28 (0.75)	2.47 (1.09)	2.28 (0.75)	2.46 (1.10)	2.55 (1.02)	167 (8, 246) <0.001

Legend Table 4.1

Abbreviations: Cc, concordant; Dc, discordant; MZ, monozygotic; DZ, dizygotic

4.3.5. Model Fitting Analysis

4.3.5.1. The Twin Model

4.3.5.1.1. Data from identical (MZ) and non-identical (DZ) twins and siblings allows the decomposition of variation in, and covariation between, traits into latent genetic and environmental factors based on their hypothetical contributions to the correlation between different types of family relationships.

4.3.5.1.2. MZ twins correlate 1 for additive genetic effects A (also known as narrow-sense heritability, h^2), representing the combined effect of alleles at, and across, loci that 'add up' to affect a behaviour, while DZ twins and siblings correlate half (i.e. 0.5). Conversely, twins and siblings correlate equally (i.e. 1.0) for shared environmental effects (C or c^2), while unique environmental effects (E or e^2) are modelled as 0. Instead of C, a non-additive genetic or dominance component (D or d^2) can be modelled, which correlates 1 for MZ twins and .25 for DZ twins and sibs and is consistent with DZ/sibling correlations that are smaller than half the MZ correlation. As a consequence of these different degrees of genetic correlations between MZ and DZ twins/siblings, but the same degree of correlation in environmental influences, a higher trait correlation in MZ than DZ twins/siblings, is assumed to represent the effects of genetic factors (Rijsdijk, van Haren et al. 2005). In addition, twin-specific environmental effects (S) were modelled, to account for the potential excess in correlation in twin pairs compared to sibling pairs.

4.3.5.2. Bivariate Modelling

4.3.5.2.1. Bivariate models estimate A, C or D, S and E of the individual variables through the MZ:DZ/sibling cross-member within-trait (Trait A or B in Subject 1 with that of their co-twin/sibling) correlations, partitioning the covariance between the two traits into A, C or D, S and E through the cross-member cross-trait (Trait A in Subject 1 with Trait B of their co-twin/sibling) MZ:DZ/sibling correlation ratios. Significant cross-member within/cross traits covariance implies that common aetiological factors between two traits are familial. These familial effects are genetic in nature if the cross-member cross-trait correlation is greater for MZ twins than for DZ/siblings and of the order 2 to 1, whereas a 1 to1 ratio suggests that shared-environmental effects (C) induce the correlation between the two traits. Non-significant cross-member cross-trait correlations suggest that the shared aetiological influences on the two traits are non-familial, i.e. due to unique environmental effects, E.

4.3.5.2.2. The partitioning of the covariation between schizophrenia and each developmental measure into genetic, shared and unique environmental sources of covariation, yields genetic (r_g), common environmental (r_c) and individual-specific environmental (r_e) correlations: r_g indicates the extent to which the same genetic

effects impact on both schizophrenia and development and personality, while the r_c reflects the degree to which the environmental effects inducing a shared environmental correlation for one trait (e.g. abnormal childhood social development) are the same with those in the second trait (e.g. schizophrenia). The level to which the unique environmental influences are common in both traits is given by the r_e.

4.3.5.2.3. As the r_g, r_c, and r_e correlations do not take into account the heritability of either trait, it is possible for a large genetic correlation to explain very little of the observed co-variation between two traits. Combining the information from the r_g, r_c, and r_e with the heritabilities, c^2 and e^2 of each trait allows us to establish the genetic ($r_{ph\text{-}a}$), common environmental ($r_{ph\text{-}c}$) and unique environmental ($r_{ph\text{-}e}$) contribution to the total phenotypic correlation (r_{ph}) between two traits.

4.3.5.3. The Fixed Bivariate Threshold Liability Model

4.3.5.3.1. In order to simultaneously analyse dichotomous diagnostic and continuous developmental data, the latter were ordinalized into five equal classes, after potential sources of variation and noise, age and gender, were partitioned out. The bivariate liability threshold model assumes a continuum of genetic risk that is normally distributed, the disorder occurring when it exceeds the liability threshold. All correlations between schizophrenia and social development and personality, both within and across individuals, were derived from the combined sample. In other words, both affected and unaffected individuals were assumed to be part of the same distribution of liability, with each individual below or above that threshold. As data were from twin pairs and siblings selected for schizophrenia rather than randomly, the sources of variance of the developmental/personality variables and their covariance with schizophrenia were estimated after ascertainment correction. This involved fixing the model parameters of the selection variable (schizophrenia) to values estimated by an earlier meta-analysis(Sullivan, Kendler et al. 2003) (h^2=0.81, c^2=0.11, e^2=0.08) and set the threshold to the population life-time prevalence (1%).

4.3.5.3.2. A goodness of model-fit index ($\chi 2$) was obtained by computing the difference in likelihoods and the degrees of freedom between the genetic AC/DSE model and a constrained correlational model in which the observed twin and sibling correlations are explained with a limited number of parameters given the complexity of the family data (Rijsdijk, van Haren et al. 2005).

4.3.5.4. Polychoric Correlations

4.3.5.4.1. To estimate the MZ and DZ/sibling correlations within and across each developmental and personality rating and schizophrenia, we fitted a constrained correlational model to the data to get: one within-member cross-trait correlation (e.g. childhood social development with liability to schizophrenia) equal across all

individuals in the sample regardless of their zygosity; one MZ, DZ and sibling cross-member within-developmental-trait correlation; one MZ, DZ and sibling cross-member cross-trait correlation. In line with the correction for selection described above, in each correlational model for schizophrenia the MZ and DZ/sibling cross-member correlations were fixed according to the point estimates of the meta-analysis: rMZ = .92 and rDZ = .515.

4.4. Results

4.4.1. Group Differences-Comparison of Means

4.4.1.1. The groups differed significantly in age, gender, years in full time education and social class (Table 4.1). Summary statistics for the developmental and personality scores are shown in Table 4.2.The subsequent group comparisons for the developmental and personality scores were adjusted for age and gender to control for potential confounding effects.

4.4.1.2. There was an overall effect of group (Table 4.2) on both childhood and adolescent social development and premorbid schizotypal ratings.

4.4.1.3. The post hoc group tests are shown in Table 4.3. There were no significant differences in the means between DZ twins and siblings for any of the developmental and personality variables. Since DZ twin pairs and sibling pairs share the same genetic relationship, we felt justified in collapsing them into single groups on the basis of concordance and diagnostic status.

4.4.1.4. Irrespective of zygosity or concordance, patients with schizophrenia were rated to have experienced more abnormal childhood and adolescent social development and more abnormal childhood personality than healthy controls.

4.4.1.5. The well co-twins from MZ discordant pairs, their co-twin had schizophrenia, were also more abnormal on all three developmental ratings compared to the healthy controls. In contrast, the well DZ co-twins and siblings from discordant pairs did not differ from controls for childhood social development and schizotypal ratings and only approached significance for adolescent social development.

Table 4.2 Summary statistics of means and standard deviations for twins & siblings

	MZ Cc ill	*Siblings Cc ill*	*MZ Dc ill*	*DZ Dc ill & Siblings Disc ill*	*MZ Dc well*	*DZ Dc well & Siblings Disc well*	*All Controls*	*Group Comparison F or χ2 (df) p*
CSA	2.35 (0.71)	2.80 (1.18)	2.51 (0.77)	2.73 (1.01)	2.31 (0.80)	2.04 (0.69)	1.71 (0.53)	41 (11, 236) <0.001
ASA	2.75 (0.89)	3.22 (1.46)	3.06 (0.98)	3.10 (1.25)	2.65 (0.97)	2.08 (0.73)	1.78 (0.61)	33 (11, 236) <0.001
PSST	1.64 (0.56)	1.47 (0.44)	1.47 (0.40)	1.41 (0.38)	1.43 (0.36)	1.13 (0.27)	1.11 (0.15)	60 (11, 247) <0.001

Abbreviations: Cc, concordant; Dc, discordant; MZ, monozygotic; DZ, dizygotic ; CSA, childhood social adjustment ; ASA, adolescent social adjustment ; PSST Premorbid Schizotypal traits

Table 4.3 Post hoc adjusted mean comparisons in twins and siblings

	MZ Cc ill vs All Controls		***MZ Dc ill vs All Controls***		***MZ Dc well vs All Controls***		***Siblings Cc ill vs All Controls***		***DZ Dc ill & Siblings Dc ill vs All Controls***		***DZ Dc well & Siblings Dc well vs All Controls***	
	t	***p***	**t**	***p***	**t**	***p***	**t**	***p***	**t**	***p***	**t**	***p***
CSA	3.61	<0.001**	4.16	<0.001**	3.03	0.003**	2.96	0.003**	6.74	<0.001**	2.14	0.033
ASA	4.79	<0.001**	5.18	<0.001**	3.50	0.001**	3.22	0.001**	6.33	<0.001**	2.65	0.009
PSST	4.00	<0.001**	3.12	0.002**	3.11	0.002**	2.85	0.005**	3.62	<0.001**	1.10	0.27

Abbreviations: Cc, concordant; Dc, discordant; MZ, monozygotic; DZ, dizygotic ; CSA, childhood social adjustment ; ASA, adolescent social adjustment ; PSST Premorbid Schizotypal traits. **significant difference at p<0.0083 Bonferroni corrected

Table 4.4 Cross-twin/sibling within trait and cross twin/sibling cross-trait correlations (r & 95% CI)

	Correlation of developmental and personality score within members of twin and sibling pairs				***Correlation of developmental and personality score with schizophrenia across members of twin and sibling pairs***			
	MZ	***DZ***	***Siblings***	***DZ & Sibs combined***	***MZ***	***DZ***	***Siblings***	***DZ & Sibs combined***
CSA	0.77 (0.59/0.87)	0.28 (-0.16/0.60)	0.28 (0.18/0.37)	0.23 (0.12/0.36)	0.39 (0.28/0.49)	-0.01 (-0.20/0.24)	0.24 (0.12/0.38)	0.25 (0.15/0.34)
ASA	0.56 (0.30/0.52)	0.13 (-0.32/0.53)	0.41 (0.28/0.54)	0.38 (0.25/0.50)	0.42 (0.31/0.52)	0.250 (-0.00/0.51)	0.30 (0.19/0.39)	0.28 (0.18/0.37)
PSST	0.75 (0.58/0.86)	0.28 (-0.13/0.59)	0.10 (-0.01/0.23)	0.11 (0.03/0.24)	0.35 (0.25/0.44)	0.11 (-0.10/0.32)	0.20 (0.11/0.29)	0.19 (0.10/0.28)

Abbreviations: MZ, monozygotic; DZ, dizygotic ; CSA, childhood social adjustment ; ASA, adolescent social adjustment ; PSST Premorbid Schizotypal traits.

Note: The schizophrenia cross-twin correlation (SZtw1–SZtw2) is constrained to be .92 in MZ twins and .515 in DZ twins based on the point estimates of meta-analysis results, and the thresholds on the liabilities are fixed to a prevalence of 1%. Intervals including 0 indicate non-significance.

Table 4.5. Additive genetic, dominant genetic, shared and specific environmental estimates (with 95% CI) of the AD/CE genetic model for CSA, ASA, and PSST

	a^2	d^2	c^2	e^2
CSA	0.17 (0.09/ 0.60)	0.63 (0.16/ 0.76)	-	0.20 (0.12/ 0.35)
ASA	0.46 (0.06/ 0.67)	-	0.16 (0.08/ 0.40)	0.39 (0.24/ 0.60)
PSST	0.13(0.065/ 0.45)	0.53 (0.17/ 0.67)	-	0.34 (0.23/ 0.51)

Abbreviations: CSA, childhood social adjustment ; ASA, adolescent social adjustment ; PSST Premorbid Schizotypal traits.

Note: a2, d2, c2 and e2: broad heritability, dominant genetic, shared and non-shared environmental. Confidence intervals including zero indicate non-significance.

Parameters for schizophrenia are fixed based on a prevalence of 1% and the following genetic model: h2=.81, c2=.11, e2=.08

Table 4.6 The phenotypic correlations between schizophrenia and the developmental and personality scores (rph), the decomposed sources of the correlations (rph-a, rph-c, rph-e) predicted by the AC/DE models and correlation estimates (with 95% CI)

	$\mathbf{r_{ph\text{-}a}}$	$\mathbf{r_{ph\text{-}c}}$	$\mathbf{r_{ph\text{-}e}}$	$\mathbf{r_{ph}}$	$\boldsymbol{r_a}$	$\boldsymbol{r_c}$	$\boldsymbol{r_e}$
CSA	0.37	-	0.003	0.37 (0.29/ 0.45)	1.0 (0.49/1.0)	-	0.03 (-0.39/0.46)
ASA	0.33	0.09	0.01	0.43 (0.35/0.51)	0.55 (0.35/1)	0.70 (-0.39/0.89)	0.03 (-0.34/0.40)
PSST	0.33	-	0.005	0.33 (0.25/ 0.41)	0.99 (0.51/1.00)	-	0.03 (-0.30/0.35)

Abbreviations: rph: total phenotypic correlation; rph-a, rph-c, rph-e, phenotypic correlation due to additive genetic, shared and unique environmental influences. ra, re, re, correlation between additive genetic, shared and specific environmental factors. Confidence intervals including zero indicate non-significance. Fixed genetic model for Schizophrenia used: h2=.81, c2=.11, e2=.08

4.4.2. Genetic Model Fitting

4.4.2.1. Table 4.4 shows the maximum likelihood polychoric correlations for the MZ and DZ twin pairs and siblings. A sub-model of the constrained correlational model showed no significant difference in DZ twins and siblings for any of the variables ($\Delta\chi$(df=2): 4.99, 1.83 and 1.75; p-values: 0.08, 0.40 and 0.42 for CSA, ASA and PSST, respectively) For CSA and PSST the pattern of MZ and DZ/sibling correlations suggested non-additive genetic effects (consequently an ADE model was fitted), whereas for ASA an ACE model was indicated, both included twin-specific environmental effects (S). Since these twin-specific effects were non-significant for all three variables ($\Delta\chi$(df=1): 0.66, 0 and 3.8; p-values: 0.41, 1 and 0.05 for CSA, ASA and PSST, respectively), we evaluated the estimates from the full AD/CE models.

4.4.2.2. Table 4.5 shows that significant additive genetic heritability (a^2) effects were found for all three variables. There was further evidence of dominant genetic (d^2) effects for childhood social development and schizoid/schizotypal personality and shared environmental effects for adolescent social development. The remainder of the variance was explained by unique environmental influences (e^2), including measurement error.

4.4.2.3. Table 4.6 shows that an increased liability for schizophrenia was associated with greater abnormalities in each of the developmental variables (r_{ph}). All three abnormal developmental markers had significant additive genetic correlations with schizophrenia (r_a),. whereas specific environmental correlations (r_e) and the shared environmental correlation in ASA (r_c) were not significant. Therefore, additive genetic factors were the main source of the phenotypic correlation between all three of the developmental variables and schizophrenia ($r_{ph\text{-}a}$).

4.5. Discussion

4.5.1. This studyconfirmed that both social development and schizotypal personality traits are abnormal in children and adolescents who subsequently develop schizophrenia, while unaffected relatives demonstrate qualitatively similar abnormalities suggesting a familial effect. Our model fitting analyses allowed us to extend this interpretation, supporting the notion that abnormal early social development and schizotypal personality traits are related to schizophrenia through common genetic influences.

4.5.2. Premorbid social behaviour abnormalities are well replicated in schizophrenia (Done, Crow et al. 1994; Malmberg, Lewis et al. 1998; Cannon, Caspi et al. 2002; Addington and Addington 2005), with little effect of gender(Olin and Mednick 1996; Cannon, Jones et al. 1997; Addington and Addington 2005). They deteriorate from

childhood through adolescence(Davidson, Reichenberg et al. 1999; Tarbox and Pogue-Geile 2008).

4.5.3. The aetiology of these deficits has in the past been unclear with genetic and environmental origins proposed(Sobel 1961; Ragins, Schachter et al. 1975; Hans, Auerbach et al. 2000; Hans, Auerbach et al. 2005; Shim, Kang et al. 2008). The core of these pre-schizophrenia social deficits centre on the quality of peer relations(Lewine, Watt et al. 1980), especially with people of the opposite sex(Dworkin, Lewis et al. 1994; Malmberg, Lewis et al. 1998), through social immaturity and peer rejection(Hans, Auerbach et al. 2000). Furthermore, the more extreme the difficulties in establishing and maintaining peer relationships, the greater the future risk for schizophrenia(Malmberg, Lewis et al. 1998).

4.5.4. Social adjustment abnormalities both in childhood (a^2=0.17) and adolescence (a^2=0.46) were heritable. The additive genetic correlations for childhood (r_a=1.0) and adolescent (r_a=0.55) social development with schizophrenia indicate that the same genetic effects contribute to variance in abnormal social development at both ages and to the liability for schizophrenia. For both childhood and adolescent social development much of this phenotypic correlation was attributable to shared additive genetic effects, environmental effects were insignificant. Correlations however do not prove causality and this study is still unable to determine if the liability to schizophrenia impairs early social development or if, from the endophenotype perspective, impaired early social development causes schizophrenia. Equally they could be linked by another common patho-physiological process.

4.5.5. Broad heritability of schizotypal traits in twin studies range between 27 and 60%, depending on the measures used, with considerable overlap of confidence intervals (Kendler and Hewitt 1992; Hay, Martin et al. 2001; MacDonald, Pogue-Geile et al. 2001). Schizotypal personality traits are, phenomenologically at least intricately linked to schizophrenia(Liddle 1987; Bentall, Claridge et al. 1988; Liddle and Barnes 1990; Bergman, Silverman et al. 2000), more prominent in men(Kremen, Faraone et al. 1998), linked with minor psychotic symptoms in childhood(Poulton, Caspi et al. 2000) and are associated with an increased future risk of schizophrenia(Vourdas, Pipe et al. 2003). They hypothetically lie on a population based continuum of psychosis(van Os, Verdoux et al. 1999); indeed the modest phenotypic correlation with schizophrenia (rph=0.33) in this study was perhaps surprising. Despite some inconsistency, studies in relatives and twins (Baron, Gruen et al. 1985; Grove, Lebow et al. 1991; Kendler, McGuire et al. 1993; Maier, Lichtermann et al. 1994; Battaglia, Bernardeschi et al. 1995; Bergman, Silverman et al. 2000) generally support familial aggregation with schizophrenia, particularly for the affective and interpersonal components (Kremen, Faraone et al. 1998),. However whether

schizotypy is a unitary(Jang, Woodward et al. 2005), or like schizophrenia, a multidimensional construct (Kendler, McGuire et al. 1995; Vollema and van den Bosch 1995; Suhr and Spitnagel 2001; Linney, Murray et al. 2003), with attendant aetiologically heterogeneity, still remains unclear.

4.5.6. Additive genetic effects accounted for 13% and dominant genetic effects 53% of the variance in schizotypal personality ratings, the results broadly speaking in line with studies that have found evidence of both additive and non-additive (dominant) genetic influences across a variety of personality domains(Keller, Coventry et al. 2005; Rebollo and Boomsma 2006; Rettew, Rebollo-Mesa et al. 2008). While the phenotypic correlation for schizotypal personality with schizophrenia was only modest (0.33), this nonetheless represented a strong additive genetic correlation r_a with schizophrenia of 0.99, emphasising the genetically linked variance between these two 'schizophrenia spectrum' disorders, and the lack of any significant unique environmental effects to link the two.

4.5.7. The influence of unique environmental factors on the premorbid markers was apparent in our sample, and contributed to developmental divergence. The majority of discordant MZ twin studies have reported that the future probands were distinguishable pre-morbidly from their genetically identical co-twins by their abnormal personality (Arieti 1949; Kringlen 1967; Mosher, Pollin et al. 1971; Fischer and Christen.A 1973), social development(Pollin, Stabenau et al. 1966), academic ability (Stabenau and Pollin 1967) and sub-clinical non-psychotic symptoms (Stabenau and Pollin 1967). Studies in unaffected first degree relatives of patients with schizophrenia (Cannon-Spoor, Potkin et al. 1982; Foerster, Lewis et al. 1991; MacDonald, Pogue-Geile et al. 2001) strongly suggest that similar, if less marked early developmental deficits are present in the unaffected relatives, supporting at the very least a familial effect.

4.5.8. This data suggest that genetically influenced abnormalities in social development and personality are linked to the genetic risk for schizophrenia, though that unique environmental influences drive up to a third of the variance in developmental and personality data in the twins and siblings, both in their social abilities and personality and later schizophrenia. Candidate environmental factors for schizophrenia include obstetric complications(McNeil, Cantorgraae et al. 1994), childhood trauma(Morgan and Fisher 2007; McEwen 2008), drug exposure(Arseneault, Cannon et al. 2002; O'Daly, Guillin et al. 2005), immigration status(Veiling, Susser et al. 2008) and social inequality(Boydell, van Os et al. 2004), each factor's influence varying with the developmental stage and possibly cumulative in effect(Cougnard, Marcelis et al. 2007). Models suggesting how these processes operate, through affective(Myin-Germeys, Krabbendam et al. 2003), cognitive, stress sensitization(Collip, Myin-Germeys et al. 2008), dopamine

sensitization(Laruelle 1998; Laruelle 2000; Featherstone, Kapur et al. 2007) or hypothalamic-pituitary-adrenal axis dysfunction(Walker and Diforio 1997; Tarbox and Pogue-Geile 2008; Walker, Mittal et al. 2008) however remain speculative.

4.5.9. Strengths and Limitations

4.5.9.1. Twin modelling is dependent on the validity of the equal environments assumption (Joseph 1998). A finding called into question in our study by the low DZ correlations, though other explanations include sibling interaction and genetic dominance and epistasis (Loehlin 1986). As has been noted earlier in this thesis (see 2.3), it is likely that the environmental experiences of MZ twins are more similar than DZ twins and siblings, though that this is in fact driven by their shared genotype (Plomin and Bergeman 1991).

4.5.9.2. While the non-psychotic co-twins and siblings in this study were at high genetic risk, they were actually at very low risk of ever developing schizophrenia, as all the non-psychotic co-twins from discordant pairs had remained free of schizophrenia for long enough after their co-twin had become ill (Belmaker, Pollin et al. 1974). Thus we can be confident that these findings are not driven by non-psychotic relatives, with an excess in the MZ twins, who will later develop schizophrenia.

4.5.9.3. This study was hampered by its retrospective design, in particular that mothers were asked to recall aspects of their children's development and personality from years earlier and after, in some cases one or more of the children had developed schizophrenia. The mothers' reports are subjective and prone to rater error and contrast bias. However the mothers of the discordant twins and siblings did not appear to have elevated their 'threshold' for normality by virtue of having an 'abnormal' child who later developed schizophrenia, leading to under reporting of abnormalities in the non-psychotic co-twins. In fact we detected the opposite, with more abnormalities reported in the unaffected co-twins and siblings than controls.

4.5.9.4. The subjects were not drawn from an epidemiological sample and while this could introduce a selection bias and inflate the genetic estimates with schizophrenia, this effect was minimised as the subjects were referred nationally by NHS psychiatrists. The NHS is comprehensive, free at the point of delivery service, it is socially inclusive and cares for the majority of patients with schizophrenia in the UK, making it a highly representative recruitment source.

4.5.9.5. As before both our control sample and well co-twins and siblings from discordant pairs, contained subjects with other lifetime axis I disorders. In order to maintain diagnostic consistency between all the non-schizophrenic subjects (discordant MZ and DZ/siblings and control MZ and DZ/siblings), I applied the same psychiatric exclusion criteria to all. Consequently these groups contained subjects with lifetime

histories of other axis I disorders though none were symptomatic at the time of testing. I considered that to introduce additional exclusion criteria for the controls, specifically that of any axis I pathology, would artificially inflate differences with the controls.

4.5.9.6. Strengths of this study include that this sample represents the largest national examination of premorbid developmental traits in twins and siblings with schizophrenia, and the only to include both MZ and DZ discordant pairs with known zygosity and clinically assessed. Genetic model fitting in a combined twin and family sample increased the power to detect genetic and environmental effects (Posthuma, de Geus et al. 2000; Boomsma, Busjahn et al. 2002), rigorous structured assessment criteria were used and improved statistical methods capitalised on the strengths of the twin/sibling model avoiding issues of independence.

4.5.10. This study supports the view that heritable, genetically determined premorbid abnormalities of social adjustment and personality are found in schizophrenia. These deficits are detectable in those who will later develop schizophrenia and their non-psychotic relatives, and reflect shared genetic risk. They thus meet many endophenotype criteria.

Chapter 5

5. Total, Grey and White Matter Cerebral Volumes in Monozygotic Twins with Schizophrenia

5.1. ABSTRACT

5.1.1. Background: Reductions in the total whole brain, grey, and to a lesser extent white matter volumes are now recognised as robust features of schizophrenia. However, it remains unclear to what extent these abnormalities are determined by genetic or environmental risk for the disorder. In this study I investigated the influence of these risk factors on brain volumes in monozygotic (MZ) twin pairs concordant and discordant for schizophrenia as well as healthy control twins.

5.1.2. Methods: Total whole brain, grey and white matter volumes were calculated from structural magnetic resonance images using an automated alogorithm implemented in SPM2 from 63 MZ twins pairs, comprising of 21 MZ pairs concordant for schizophrenia, 17 MZ pairs discordant for schizophrenia, and 25 healthy MZ twin pairs.

5.1.3. Results: Whole brain, grey and white matter volumes were all smaller in probands with schizophrenia, while there were no significant differences between probands with schizophrenia whether they came from concordant or discordant pairs. Well co-twins from discordant pairs differed significantly on all three volumes compared to the healthy controls, though these findings did not survive correction for multiple testing. The ill and well co-twins from discordant pairs did not differ from each other in any of the three volumes. There was a negative association for all three volumes with abnormal childhood developmental markers.

5.1.4. Conclusions: Gross cerebral volume reduction in schizophrenia, across white and grey matter, is determined primarily by the familial, and indeed probably the genetic risk for disorder rather than by unique environmental effects.

5.2. Introduction

5.2.1. Structural neuroimaging studies of schizophrenia have robustly shown that the illness is associated with reductions in total cerebral and grey matter volumes (Wright, Rabe-Hesketh et al. 2000{Shenton, 2001 #49; Shenton, Dickey et al. 2001; Honea, Crow et al. 2005), with more limited and indeed variable evidence of white matter volume changes (Cannon, van Erp et al. 1998; Lawrie and Abukmeil 1998; Hulshoff Pol, Schnack et al. 2002; Honea, Crow et al. 2005).

5.2.2. The pathophysiological significance of these volumetric deficits remains contentious. For instance, whether they represent static markers of early abnormal neurodevelopment, facets of later neurodegeneration, or a combination of the two

(Woods 1998; Lieberman 1999; Lieberman 1999) is unresolved. Furthermore it is possible that other factors related to the illness, for instance its treatment with antipsychotic medication, may have additional effects on these tissue volumes (Lieberman, Tollefson et al. 2005). Finally, from a causative perspective, given schizophrenia's aetiological complexity (Picchioni and Murray 2007; van Os and Kapur 2009), the extent to which these structural abnormalities are driven by the genetic liability (Sullivan, Kendler et al. 2003) as opposed to environmental factors (van Os, Kenis et al. 2010), is poorly understood.

5.2.3. The presence of qualitatively similar volumetric deficits in patents with schizotypal personality disorder (Dickey, McCarley et al. 2001; Dickey, McCarley et al. 2007; Goldman, Pezawas et al. 2009; Goldstein, Hazlett et al. 2009) and patients' non-psychotic relatives, including parents, siblings and offspring, has suggested that at least some of these abnormalities are related to the familial, and by implication the genetic risk for the disorder (Keshavan, Montrose et al. 1997; Sharma, Lancaster et al. 1998; Staal, Pol et al. 2000; Keshavan, Dick et al. 2002; Whalley, Whyte et al. 2005). However contradicting this, a recent large study of over 200 siblings of schizophrenia patients, found no significant differences in grey matter volume and density, compared to controls. The authors concluded that the structural brain changes seen in schizophrenia were unlikely to be due to genetic factors (Goldman, Pezawas et al. 2008; Honea, Meyer-Lindenberg et al. 2008).

5.2.4. Family studies alone cannot discriminate between competing genetic and shared environmental factors. Studies of monozygotic (MZ) twins, concordant and discordant for schizophrenia, are one means of addressing some of the uncertainty around the genetic influence on brain structure in schizophrenia (Reveley, Reveley et al. 1982; Suddath, Christison et al. 1990; Bartley, Jones et al. 1997; Sharma, Lancaster et al. 1998; Baare, van Oel et al. 2001; Cannon, Thompson et al. 2002; Hulshoff Pol, Brans et al. 2004; van Erp, Saleh et al. 2004; van Haren, Picchioni et al. 2004; Rijsdijk, Van Haren et al. 2005; Hulshoff Pol, Schnack et al. 2006; Ettinger, Picchioni et al. 2007; Koolschijn, van Haren et al. 2008; Ettinger, Schmechtig et al. 2010).

5.2.5. However, to date, the findings from volumetric studies of such twin pairs have in some respects been inconsistent. The majority of studies have adopted a region of interest approach and found that twins with schizophrenia have larger lateral ventricles (Reveley, Reveley et al. 1982; Reveley, Reveley et al. 1984), and smaller total grey matter volume than their non-psychotic co-twins. They have also found smaller hippocampal and hypothalamic volumes (Suddath, Christison et al. 1990; Baare, van Oel et al. 2001; Hulshoff Pol, Brans et al. 2004; Koolschijn, van Haren et al. 2008) and smaller thalamic and frontal volumes (Ettinger, Picchioni et al. 2007; Ettinger,

Schmechtig et al. 2010). Taken together these findings tend to implicate unique environmental effects driving these volume reductions, with variable evidence of genetically determined deficits in the well co-twins.

5.2.6. There are three studies that adopted a whole brain approach to map regional grey matter volume differences in discordant twins (Cannon, Thompson et al. 2002; Hulshoff Pol, Schnack et al. 2006; Borgwardt, Picchioni et al. 2010). Unfortunately each adopted a different analytical approach, and their results are inconsistent, with a mixture of familial (shared environment and genetic), genetic, and unique environmental deficits implicated principally in deficits in the frontal and temporal lobes.

5.2.7. This is the first study to examine whole brain, grey and white matter volumes to include an MZ concordant twin sample. Hypothetically such twin pairs might carry a greater genetic load for schizophrenia than those from discordant pairs (see 2.4.4.2). There is some early evidence that twins with schizophrenia from concordant compared to discordant pairs exhibit greater deficits in thalamic and some frontal volumes (Ettinger, Picchioni et al. 2007; Ettinger, Schmechtig et al. 2010).

5.2.8. In this study I used an automated technique to compare subjects' whole brain, total grey and total white matter volumes, allowing the entire brain to be examined in an objective manner without observer bias. On the basis of previous studies in schizophrenia I hypothesised that:

5.2.8.1. twins with schizophrenia would show reduced total, grey and white matter volumes relative to controls

5.2.8.2. that twins from concordant pairs would be more severely effected, with greater loss of cerebral volume

5.2.8.3. that the well co-twins from discordant pairs would occupy an intermediate position, reflecting the influence of familial effects but spared unique environmental effects

5.2.8.4. that the volumetric differences observed would correlate with abnormal childhood social development and premorbid schizotypal traits, neurodevelopmental measures that I have shown in Chapter 4 act as indices of the genetic risk for schizophrenia.

5.3. Methods

5.3.1. Recruitment

5.3.1.1. Probands, unaffected co-twins and healthy controls were recruited as described in 3.3.1.

5.3.1.2. Exclusion criteria for all subjects were as described in 3.3.1 but include a history of neurological illness or of systemic illness with known neurological

complication, a history of head injury with loss of consciousness of more than 1 minute, any current substance misuse or dependence, including alcohol and any contraindications to MR scanning.

5.3.1.3. Local and Multi-Centre ethical approval was granted. After a complete verbal and written description of the study all subjects gave written informed consent.

5.3.2. Clinical Assessment

5.3.2.1. All of the subjects were clinically assessed as described in 3.3.2. In summary clinical diagnoses were established using the Schedule for Affective Disorders and Schizophrenia—Lifetime Version augmented with further clinical information to make DSM-IV diagnoses (Spitzer, Endicott et al. 1978). Psychotic symptoms were assessed with the Scales for the Assessment of Positive (SAPS) and Negative (SANS) Symptoms (Andreasen 1983), while premorbid developmental markers were assessed using the Premorbid Social Scale (Cannon-Spoor, Potkin et al. 1982) and the Pre-morbid Assessment of Schizoid and Schizotypal Traits (Foerster, Lewis et al. 1991) from the volunteers' mothers where possible. Handedness was determined using the Annett scale (Annett 1970). Probands' medication status was recorded at assessment and converted to chlorpromazine (CPZ) equivalents (Rey, Schulz et al. 1989; Bezchlibnyk-Butler, Jeffries et al. 1994; Woods 2003; Bazire 2007; Taylor, Paton et al. 2009).

5.3.3. Participants

5.3.3.1. Structural magnetic resonance images of 126 MZ twins were obtained: 21 MZ pairs concordant for schizophrenia, 17 MZ pairs discordant for schizophrenia, and 25 healthy MZ twin pairs

5.3.3.2. The minimum time since schizophrenia onset in the probands from the discordant pairs was 4 years. Thus the probability that any of the discordant pairs would become concordant in the future was low (Belmaker, Pollin et al. 1974).

5.3.3.3. Some of the non-psychotic co-twins from the discordant pairs had a personal history of psychiatric disorders other than psychosis or schizophrenia spectrum disorders. Controls were free of any personal or family history of any psychotic illness or any schizophrenia spectrum disorder, but were still included if they had a personal history of other axis I pathology. I chose to apply the same inclusion/exclusion criteria to the controls as the non-psychotic co-twins so as not to artificially increase the differences between these two groups.

5.3.3.4. All subjects were clinically stable at the time of assessment, with no recent changes in their medications.

5.3.4. Magnetic Resonance Imaging

5.3.4.1. Participants had a structural MRI scan on a General Electric Signa Advantage scanner at 1.5 Tesla. We collected a 3-dimensional T1-weighted, coronal, spoiled gradient (SPGR) of the whole head (TE=5ms, TR=35ms, flip angle=30°, NEX=1, FOV=200x200mm, voxel dimensions = 1x1x1.5mm), yielding 124 contiguous slices 1.5mm thick. Imaging took place on identical scanners with identical protocols at either one of two sites (St Georges Hospital, London, or The Maudsley Hospital, London). Both members of every pair were scanned at the same site.

5.3.4.2. Nine twin pairs were scanned at both sites to allow comparison between sites, four MZ Concordant, three MZ Discordant and two MZ Control pairs.

5.3.4.3. *Image pre-processing*

5.3.4.3.1. Images were first evaluated by a Consultant Neuroradiologist to exclude clinically relevant pathology. All images were visually inspected for movement artefacts and converted to ANALYZE format (Robb et al., 1990). Optimised VBM pre-processing was performed with Statistical Parametric Mapping software (SPM2, http://www.fil.ion.ucl.ac.uk/spm/software/spm2/). The image processing steps have been described in detail by the developers and I will describe them only briefly here (Ashburner and Friston 2000; Good, Johnsrude et al. 2001).

5.3.4.3.2. The segmentation algorithm implemented in SPM incorporates an a priori knowledge of the likely spatial distribution of tissue types in the brain with prior probability tissue maps derived from a large number of subjects. To ensure accurate segmentation and to avoid bias during the registration step that could favour one of the groups, we created study-specific customized prior probability maps based on all subjects (Shen, Szameitat et al. 2007).

5.3.4.3.3. The pre-processing stages were as follows: 1) scans were segmented into probabilistic maps of grey and white matter and cerebrospinal fluid with a modified mixture model clustering algorithm; 2) the segmented grey matter map was mapped to the study specific grey matter template, and the derived warping parameters applied to the original T1-weighted image to map it into standard space; 3) the registered image was then re-segmented as the a priori knowledge incorporated into the SPM2 segmentation algorithm means that it works optimally in standard space. Inhomogeneity correction was performed with SPM2's bias correction algorithm. The segmented maps were then modulated through multiplying voxel values by the Jacobian determinants from the spatial normalisation to correct for volume changes. Finally, all normalised, segmented, modulated tissue maps were smoothed with an 8 mm full width at half maximum (FWHM) Gaussian filter. This smoothing kernel made the data conform to the Gaussian field model underlying the

statistical inferences implemented in SPM2 (Ashburner and Friston 2000). Also, according to the matched filter theorem, the width of the smoothing kernel determines the scale for which volumetric abnormalities will be most sensitively detected (White, O'Leary et al. 2001). Segmentation and warping accuracy was assessed by visually inspecting axial slices of each subject's GM image.

5.3.4.4. *Statistical Analysis of Total, Grey and White Matter Volumes*

5.3.4.4.1. Total raw grey and white matter volumes were calculated using the algorithms implemented in SPM (http://dbm.neuro.uni-jena.de/vbm/vbm2-for-spm2/calculate-raw-volumes). The volumes were corrected for size changes induced by spatial normalisation and summed to produce an estimate of the total brain volume.

5.3.5. Statistical analysis

5.3.5.1. *Two Site MRI Data*

5.3.5.1.1. In order to determine the presence of and address any systematic differences in the MR volumetric data between the two scanner sites I first calculated intra class correlation coefficients, then estimated the inter-site bias (Bland and Altman 1986) for each brain volume. The method identifies one site, the Maudsley, as the reference site and estimates inter-site bias, and thus the inter-site correction factor, as the mean difference between the two sites for each volume from the eighteen subjects scanned at both sites.

5.3.5.1.2. The site-specific correction factor for each volume was then applied to data from the second site, St George's Hospital.

5.3.5.2. *Twin Analysis*

5.3.5.2.1. As described in 3.3.5.2 the genetic relatedness of twin pairs and the within-family correlations violate the assumption of independent observations made in analysis of variance. Therefore, differences between groups were analysed with regression models that allowed for correlations within twin clusters and departures from normality using the robust sandwich estimator to estimate standard errors implemented in Stata 10 (Stata Corporation, College Station, TX).

5.3.5.2.2. Group differences in age, height, parental social class, years of education, premorbid schizotypy and premorbid social development, were examined using linear regression models with robust standard errors. Group differences in gender, handedness and ethnicity were examined by logistic regression with robust standard errors of the respective binary dependent variables.

5.3.5.2.3. To explore for differences in clinical data, the two proband groups (concordant and discordant ill) were compared on age at first contact with psychiatric

services, total positive (SAPS) and negative (SANS) score at time of scanning, and chlorpromazine equivalents using regression models with robust standard errors, and on type of antipsychotic medication (first or second generation) using logistic regression with robust standard errors. All groups were compared on the premorbid schizotypal and social development scores using the same statistical model.

5.3.5.2.4. For the three brain volumes, intra-class correlation co-efficients were calculated separately for each twin type (concordant, discordant and control).

5.3.5.2.5. For group comparisons of total cerebral, grey and white matter volumes, the level of significance for the analysis of the three ROIs was adjusted using the Bonferroni method (p=0.05/3=0.017). If a group comparison for a given ROI was statistically significant at this level it was followed by four planned pairwise post-hoc comparisons determined by my experimental hypotheses using a Bonferroni correction (p=0.05/4=0.0125). In order to adopt a parsimonious approach, all contrasts were adjusted for age, gender, and scanner as covariates in the models.

5.3.5.2.6. Finally clinical correlates of the brain volumes were investigated using a regression model with robust standard errors predicting brain volume in separate analyses from childhood and adolescent social development and schizotypal ratings in all subjects, and SAPS, SANS in the patients, using the same covariates. For each volume, Bonferroni correction was applied for five contrasts with a threshold of p=0.05/5=0.01.

5.4. Results

5.4.1. Demographic and clinical variables

5.4.1.1. Comparable numbers of twins were scanned at each site (χ^2=1.59, df=3, p=0.66). There was no significant difference in group membership, i.e. whether MZ twins were from concordant, discordant or control pairs, between the two scanner sites (χ^2 =3.91 (3) p=0.27).

5.4.1.2. The demographic and clinical variables are summarised in Tables 5.1 and 5.2. There were no significant effects of group on age, gender, handedness, ethnicity, height, parental social class, or years of education. Among the non-psychotic members of the discordant pairs, eleven met criteria for a previous DSM-IV Axis I disorder: depression (n=3); depression and alcohol abuse (n=1); depression and simple phobia (n=1); obsessive-compulsive disorder, depression and alcohol abuse (n=1); panic disorder, depression and mania (n=1); simple phobia (n=1); simple phobia, panic disorder and depression (n=1); generalised anxiety disorder and panic disorder (n=1); and generalised anxiety disorder, panic disorder and depression (n=1). Eight of the control twins met criteria for a previous DSM-IV Axis I diagnosis: depression (n=3), mania (n=1),

depression and drug and alcohol abuse (n=2), drug abuse (n=1), and specific phobia, agoraphobia and drug abuse (n=1). The frequency of psychiatric diagnoses was significantly higher in the discordant unaffected group than in the controls (χ^2=9.36, df=1, p=0.002). None of the non-schizophrenic twins were unwell at the time of assessment nor were any taking any psychotropic medication.

5.4.1.3. Table 5.2 summarises the clinical variables. In this sample, patients from concordant and discordant pairs did not differ on the proxy measures of illness severity, age at first contact with psychiatric services, or positive (SAPS) and negative (SANS) psychotic symptom scores at the time of assessment. They also did not differ in the type or total daily dose of antipsychotics, though there was a trend for the discordant ill group to be more likely to be prescribed a second generation drug. As anticipated the four groups differed on premorbid schizotypal traits and difficulties in social adjustment during childhood and adolescence, the patients had experienced more problems.

Table 5.1

Demographics of Experimental Groups

	MZ Concordant	MZ Discordant Ill	MZ Discordant Well	MZ Control	Analysis F or X2 (df) p
Number of Subjects (Pairs)	42 (21)	17 (17)	17 (17)	50 (25)	
Age	34.6 (8.4)	31.4 (13.3)	31.5 (12.0)	36.1 (10.0)	0.68 (3,64) 0.57
Gender % Female	21	35	41	32	3.36 (3) 0.34
Handedness % Right	81	81	81	82	0.03 (3) 0.99
Ethnicity % Caucasian	95	88	88	100	1.06 (2,63) 0.37
Height cm	174.5 (8.0)	175.6 (9.3)	172.1 (8.6)	174.0 8.7)	0.81 (3,61) 0.49
Social Class Parents	2.5	2.4	2.4	2.7	0.75 (3,62) 0.53
Education Years	13.7 (2.8)	12.6 (3.0)	12.7 (2.4)	14.0 (2.7)	1.36 (3,64) 0.26

Legend Table 5.1

Data reflect mean (and standard deviation) unless otherwise stated.

Table 5.2

Clinical Ratings of Experimental Groups

	MZ Concordant	MZ Discordant Ill	MZ Discordant Well	MZ Control	Analysis F or X2 (df) p
Age at First Contact	21.8 (6.0)	21.3 (6.0)	n/a	n/a	0.13 (1, 35) 0.72
SAPS	6.0 (3.6)	6.1 (3.9)	n/a	n/a	1.35 (1,34) 0.25
SANS	9.3 (3.9)	8.00 (6.0)	n/a	n/a	1.35 (1,34) 0.25
Type of Medication N Second/First Generation/na	17/16/9	9/3/5	n/a	n/a	3.55 (1) 0.06
Medication CPZ Equivalence	630 (477)	500 (371)	n/a	n/a	0.22 (1,37) 0.64
Schizotypy	1.7 (0.5)	1.4 (0.4)	1.4 (0.4)	1.0 (0.1)	10.4 (3,33) 0.0001
Childhood Social Adjustment	2.5 (0.8)	2.4 (0.5)	2.2 (0.6)	1.4 (0.3)	12.4 (3,29) <0.0001
Adolescent Social Adjustment	2.6 (0.7)	2.9 (0.6)	2.5 (0.7)	1.4 (0.3)	27.8 (3,29) <0.0001

Legend Table 5.2

Data reflect mean (and standard deviation) unless otherwise stated. SAPS=Scale for the Assessment of Positive Symptoms; SANS=Scale for the Assessment of Negative Symptoms; CPZ=chlorpromazine; n/a = not available

5.4.2. Between Scanner Site Comparison

Table 5.3

Intra Class Correlations for Whole Brain, Grey and White Matter Volume

Brain Volume	Intra Class Correlation n=18
Whole Brain	0.958
Grey Matter	0.929
White Matter	0.846

5.4.2.1. For the eighteen subjects scanned at both sites there were strong relationships between the sites for the estimates of each of the three cerebral volumes. Intraclass correlations for whole brain, grey and white matter were 0.96, 0.93 and 0.85 respectively (Figures 5.1 and 5.2). Figure 5.3 shows the Bland Altman plots for each volume. The graphs plot the difference between each site's estimate against their mean, giving an index of any systematic intersite bias (Bland and Altman 1986). The bias, defined as the mean difference were estimated for each volume and subtracted from the second site estimates. The bias estimates were: for whole brain volume = -16.7mls, for grey matter = -2.5mls and for white matter = -14.2mls.

Figure 5.1

Plot of Whole Brain Volume St George's against The Maudsley Sites

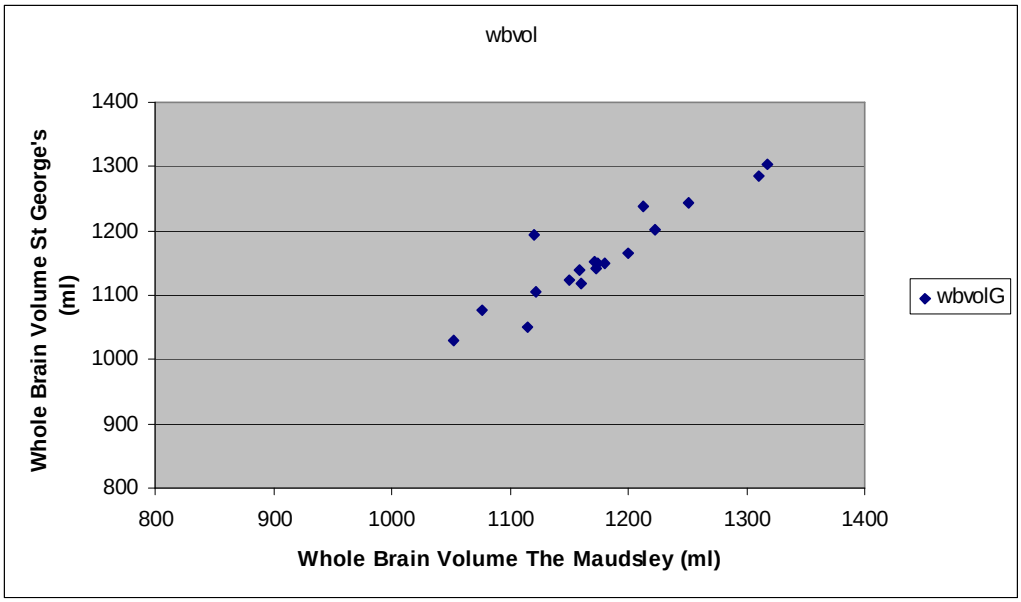


Figure 5.2

Plot of Grey and White Matter Volumes St George's against The Maudsley Sites

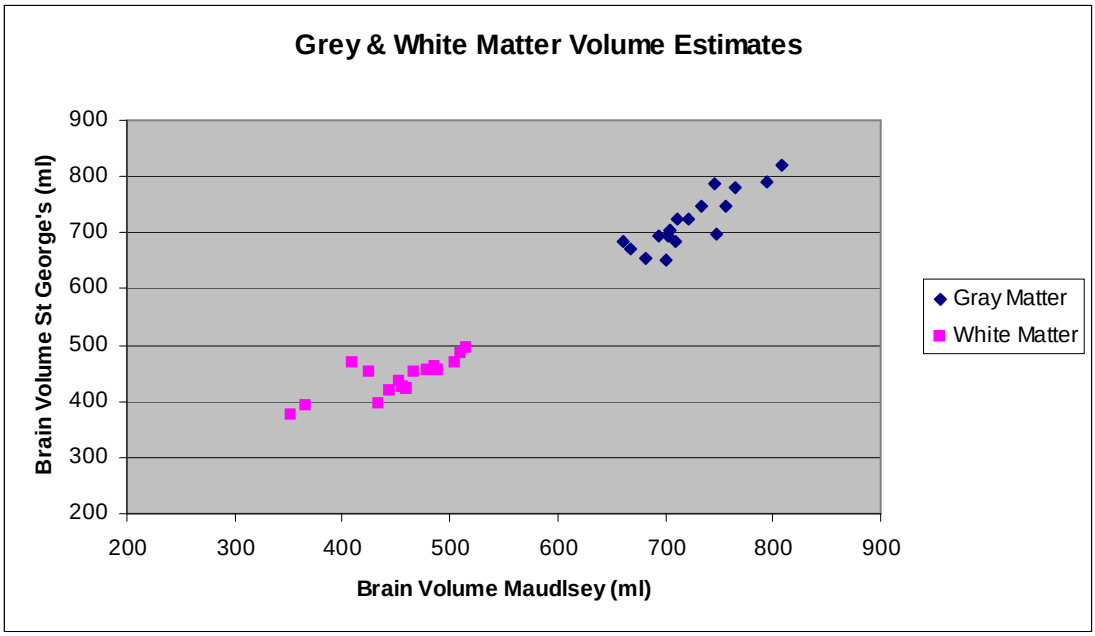
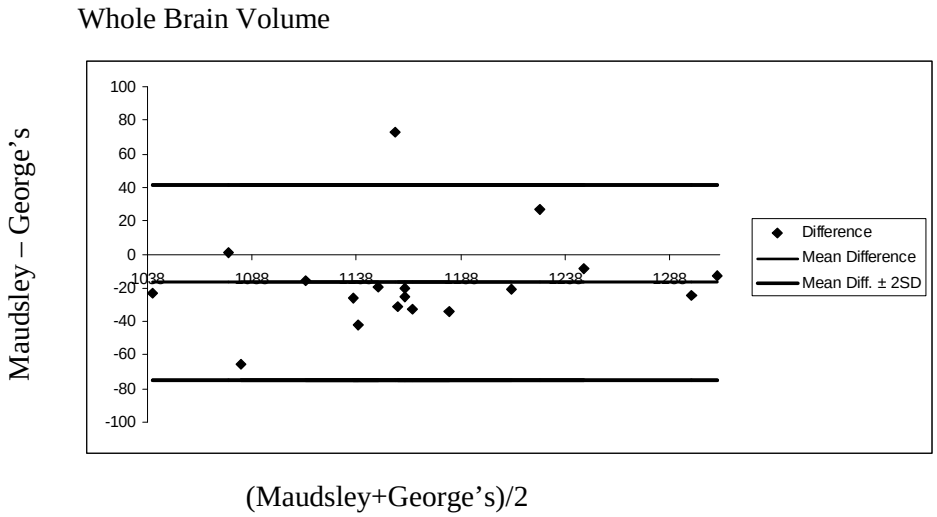
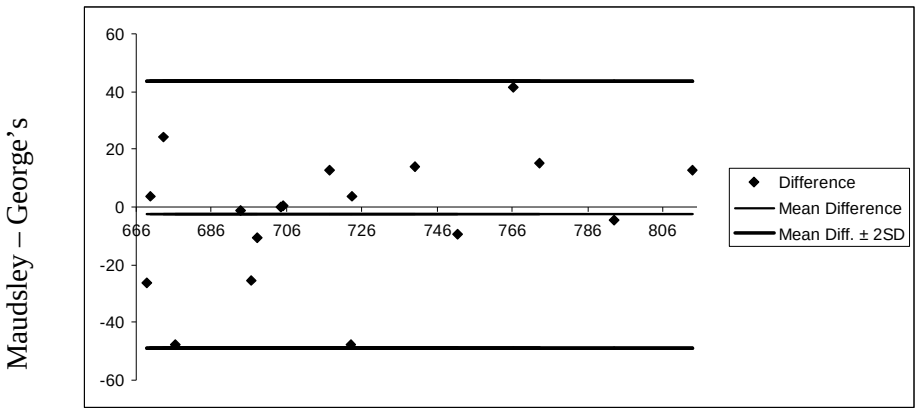


Figure 5.3

Bland-Altman Plots of Whole Brain, Grey & White Matter Volumes St George's against The Maudsley Sites

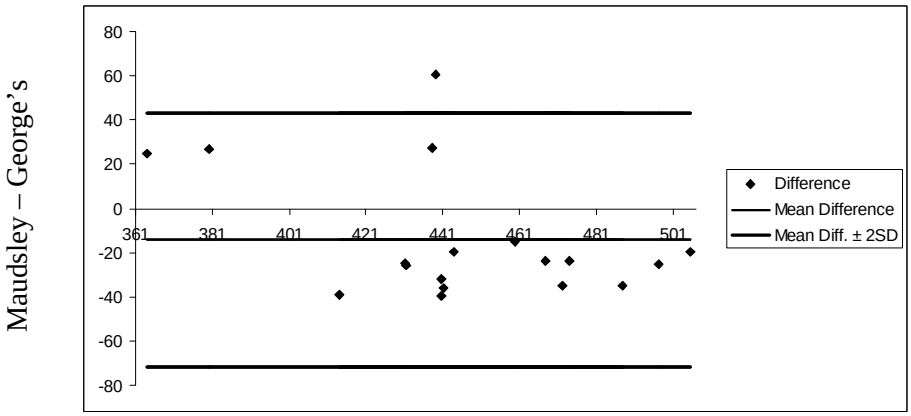


Grey Matter Volume



(Maudsley+George's)/2

White Matter Volume



(Maudsley+George's)/2

5.4.3. Brain Volumes

5.4.3.1. The adjusted mean brain volumes and their standard deviations are shown in table 5.4 and figures 5.4 to 5.6.

Table 5.4

Adjusted Brain Volumes by Group

Volume Mean (SD) ml	Group			
	MZ Concordant	MZ Discordant Ill	MZ Discordant Well	MZ Control
Whole Brain	1150.4 (96.4)	1147.2 (156.1)	1146.3 (151.8)	1207.5 (92.7)
Grey	700.2 (58.7)	711.2 (100.2)	718.0 (90.4)	750.0 (55.5)
White	450.3 (45.5)	435.9 (67.2)	428.3 (66.6)	457.6 (47.2)

Legend Table 5.4

Note: Data reflect mean (and standard deviation) of adjusted brain volumes in ml by Group. Whole Brain = whole brain volume; Grey = grey matter volume; White = white matter volume.

Figure 5.4

Mean Adjusted Whole Brain Volume by Group

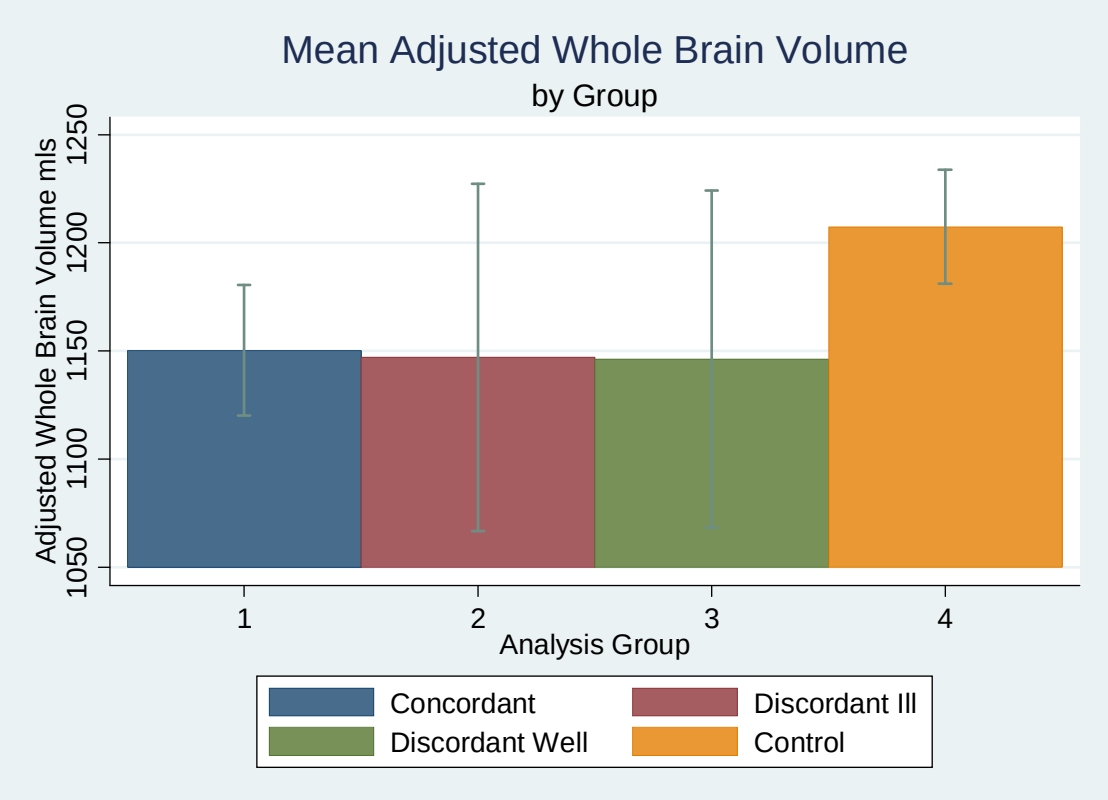


Figure 5.5

Mean Adjusted Grey Matter Volume by Group

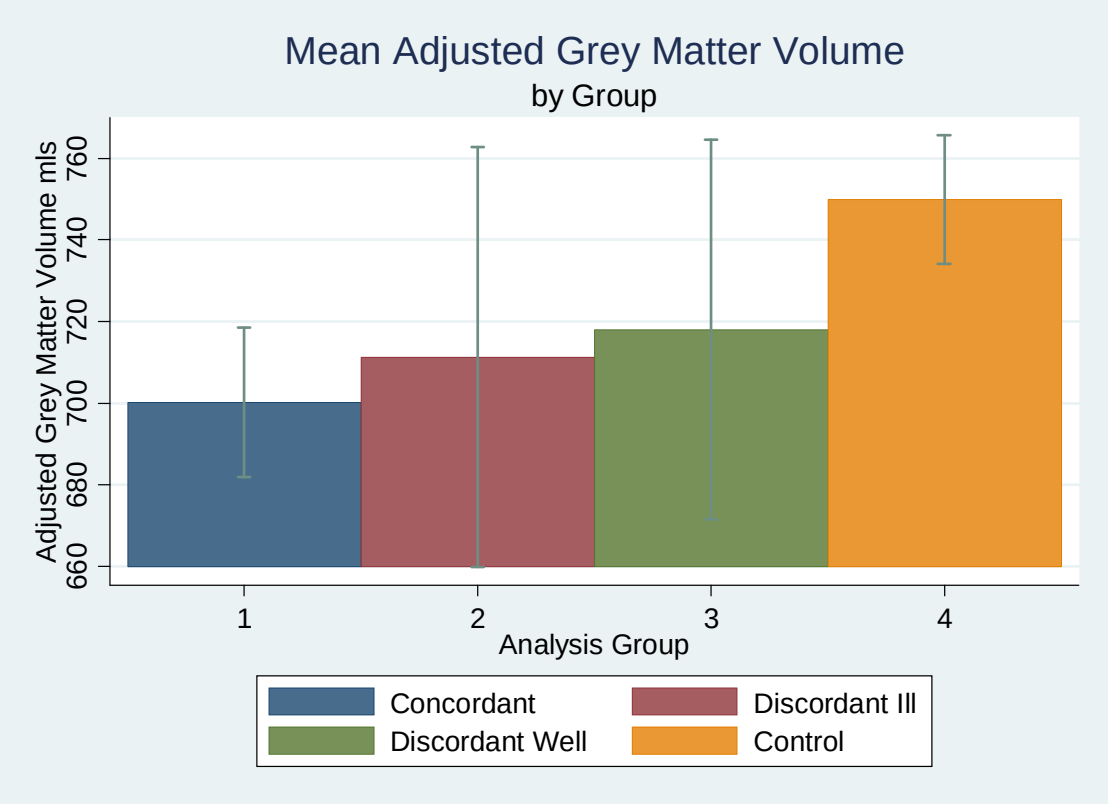
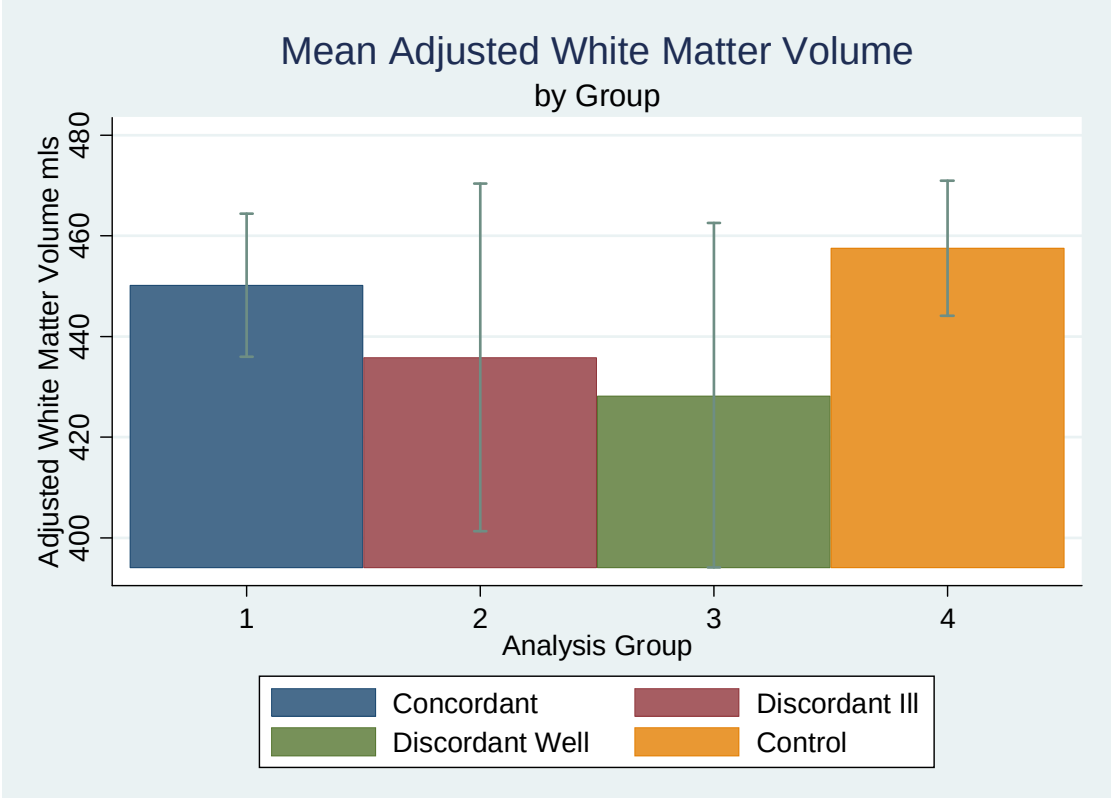


Figure 5.6

Mean Adjusted White Matter Volume by Group



5.4.3.2. *Group Differences in Whole Brain, Grey and White Matter Volumes*

5.4.3.2.1. Table 5.5 shows the results of the statistical contrasts between groups. There were significant overall effects of group on the whole brain ($F[7,64]=2153.2$, $p<0.0001$), grey ($F[7,64]=2183.4$, $p=0.001$) and white matter volumes ($F[7,64]=1611.8$, $p<0.0001$. Subsequent post-hoc comparisons revealed a similar pattern of deficits for each of the three volumes.

5.4.3.2.2. For whole brain volume, I found that patients as a whole ($t=-3.21$, $p=0.002$), and when subdivided into concordant ($t=-3.24$, $p=0.002$) and discordant ($t=-2.44$, $p=0.017$) groups, had smaller volumes than the healthy controls. Patients from concordant and discordant pairs did not differ from each other ($t=-0.02$; $p=0.99$). Discordant schizophrenic twins ($t=-2.43$, $p=0.018$) and their non-schizophrenic co-twins ($t=-2.39$, $p=0.02$) both differed from healthy control twins, though neither contrast survived stringent multiple testing correction. Finally within the MZ discordant pairs the patients did not differ significantly from their well co-twins ($t=-0.77$; $p=0.45$).

5.4.3.2.3. For grey matter, patients from concordant and discordant pairs did not differ ($t=-0.66$; $p=0.51$). Patients as a whole ($t=-3.44$, $p=0.001$), and when subdivided into those from concordant ($t=-3.79$; $p<0.001$) and from discordant pairs ($t=-2.37$; $p=0.021$) and their unaffected co-twins ($t=-2.18$; $p=0.033$) had smaller grey matter volumes than healthy controls, though neither of the last two contrasts survived multiple testing correction. The within discordant pair contrast was again not significant ($t=-1.23$, $p=0.23$).

5.4.3.2.4. For white matter, patients from concordant and discordant pairs did not differ ($t=0.77$; $p=0.44$). Patients as a whole ($t=-2.31$, $p=0.024$) differed from controls, but did not survive correction. Patients from discordant pairs ($t=-2.02$; $p=0.048$) and their unaffected co-twins ($t=-2.30$; $p=0.025$) had smaller white matter volumes than healthy controls, though neither contrast survived multiple testing correction. The contrast between the patients and unaffected co-twins within discordant pairs was again not significant ($t=0.34$, $p=0.73$).

Table 5.5 Between Group Tests

Covariates: Age, Gender & Scanner		MZ Group Comparison			
		MZ Conc vs MZ Disc Ill	**All MZ Patients vs MZ Control**	**MZ Disc Ill vs MZ Disc Well**	**MZ Disc Well vs MZ Control**
Whole Brain Volume	Group Comparison	F(7, 64) = 2153.2, p< 0.0001**			
	Pairwise Test	t=-0.02 p=0.99	t=-3.21 p=0.002***	t=-0.77 p=0.45	t=-2.39 p=0.02*
	Effect Size (95% CI)	-0.49 -58.6 to 57.6	-78.0 -1126.6 to -29.4	-7.4 -26.8 to 11.9	-70.3 -129.1 to-11.5
Grey	Group Comparison	F(7, 64) = 2183.4, p< 0.0001**			
	Pairwise Test	t=-0.66 p=0.51	t=-3.44 p=0.001***	t=-1.23 p=0.23	t=-2.18 p=0.033*
	Effect Size (95% CI)	-11.8 -47.7 to 24.1	-53.0 -83.8 to -22.2	-9.6 -25.2 to 6.0	-37.5 -71.9 to -3.1
White	Group Comparison	F(7, 64) = 1611.8, p< 0.0001**			
	Pairwise Test	t=0.77 p=0.44	t= -2.31 p=0.024*	t=0.34 p=0.73	t=-2.30 p=0.025*
	Effect Size (95% CI)	11.3 -17.8 to 40.4	-25.0 -46.7 to -3.4	2.1 -10.4 to 14.7	-32.8 -61.4 to -4.3

Legend Table 5.5

MZ Conc = MZ Concordant; MZ Disc Ill = MZ Discordant Ill; MZ Disc Well= MZ Discordant well. Whole Brain = whole brain volume; Grey = grey matter volume; White = white matter volume. *p<0.05, ** p<0.017, *** p<0.0125 corrected for multiple comparisons.

5.4.3.3. *Intra Twin Class Correlations of ROI Volumes*

5.4.3.3.1. The ICCs for the concordant, discordant and control twins for each of the three cerebral volumes are shown in Table 5.6. The ICCs were high ranging from 0.76 to 0.97. While the ICC estimates were lowest within the concordant twins and highest for the discordant twins, the confidence limits of all three groups overlapped considerably, suggesting that there were no systematic differences in their magnitude.

Table 5.6

Intraclass Correlations (ICC) by Twin Category

Volume	Group		
	MZ Concordant	MZ Discordant	MZ Control
Whole Brain	0.79 (0.63/0.96)	0.97 (0.95/0.99)	0.93 (0.88/0.99)
Grey	0.80 (0.64/0.96)	0.96 (0.91/0.99)	0.88 (0.78/0.97)
White	0.76 (0.59/0.95)	0.94 (0.88/1.00)	0.90 (0.82/0.97)

Legend Table 5.6

Intraclass correlations (ICC) coefficients (and 95% confidence interval) by twin category. Whole Brain = whole brain volume; Grey = grey matter volume; White = white matter volume.

5.4.3.3.2. *Clinical Correlates of Brain Volume*

5.4.3.3.2.1. Across the four groups of subjects there were significant associations of total cerebral, grey and white matter volumes with childhood (all p<0.0019) and adolescent (all p<0.0033) social development problems. Furthermore there were significant associations between all three volumes and the premorbid schizotypal ratings (all p<0.0001). All contrasts survived correction. On each occasion, a greater severity of developmental abnormality in childhood or adolescence was associated with smaller brain volumes in adulthood.

5.4.3.3.2.2. Within the patients there were no significant associations between any of the three brain volumes, total, grey and white matter, and positive or negative psychotic symptoms at the time of scanning (all $p>0.08$). Similarly there was no relationship between any of the three brain volumes and antipsychotic dose at the time of scanning (all $p>0.62$).

5.5. Discussion

5.5.1. In this study I compared unbiased estimates of total brain, grey and white matter volumes in MZ twins who varied in their concordance for schizophrenia. The aim was to identify the influence of familial, genetic and unique environmental factors on any brain volume deficits detected.

5.5.2. I extended the findings of our own earlier structural studies in twins with schizophrenia (van Haren, Picchioni et al. 2004; Rijsdijk, Van Haren et al. 2005; Ettinger, Picchioni et al. 2007; Borgwardt, Picchioni et al. 2010; Ettinger, Schmechtig et al. 2010), by using an objective technique to the entire brain and expanding the sample size. This was also the first study to quantify whole brain, grey and white matter volumes over the entire brain that included an MZ concordant sample.

5.5.3. I found evidence of deficits in whole brain, grey and white matter volume in the patients as well as the unaffected co-twins from discordant pairs, though the latter did not survive stringent correction for multiple testing.

5.5.4. I did not find any evidence of significant volumetric differences between members of discordant pairs, and found high correlations within twin pairs of all types for all of the tissue volumes.

5.5.5. Finally across the experimental groups there was evidence of an association between lower brain volume and developmental markers of the genetic risk for schizophrenia. There was no relationship with 'current' symptomatology or treatment in the patients.

5.5.6. Structural Deficits in Schizophrenia

5.5.6.1. Numerous structural imaging studies (Mathalon, Sullivan et al. 2001; Shenton, Dickey et al. 2001; Wood, Velakoulis et al. 2001; Kubicki, Shenton et al. 2002; Niznikiewicz, Kubicki et al. 2003), including meta analyses (Wright, Rabe-Hesketh et al. 2000; Arnone, Cavanagh et al. 2009), have shown robust evidence of volumetric differences between patients with schizophrenia and healthy controls. These deficits have been most reliably demonstrated in frontal and temporal lobe grey matter (Honea, Crow et al. 2005). Studies of white matter, whether assessing its volume and density using structural MRI, or its integrity using diffusion tensor imaging (DTI), have

tended to produce more mixed results (Kanaan, Kim et al. 2005; Kubicki, McCarley et al. 2007).

5.5.6.2. Data from this study, comparing the twins with schizophrenia and the healthy twins produced results that were entirely consistent with the established structural imaging findings, including work in twins (Hulshoff Pol, Brans et al. 2004; van Haren, Picchioni et al. 2004; Rijsdijk, van Haren et al. 2005).

5.5.7. Familial Risk Effects in Schizophrenia

5.5.7.1. Both twins with schizophrenia and their genetically identical but non-psychotic co-twins showed reductions in whole brain, grey and white matter volumes relative to healthy twins, though this did not survive stringent multiple testing correction. If accepted, this result is consistent with a familial effect, mediated through genetic and shared environmental factors. Three further lines of evidence support the conclusion that these represent genuine deficits, principally mediated by genetic effects.

5.5.7.1.1. Firstly there were no significant differences between the twins with schizophrenia and their unaffected co-twins from discordant pairs for any of the three volumes.

5.5.7.1.2. Secondly there were high correlations across all three cerebral volumes within each of the three twin groups, MZ concordant, MZ discordant and MZ control. Imaging studies in healthy twins have estimated the heritability of whole brain volume (Tramo, Loftus et al. 1995; Bartley, Jones et al. 1997; Bonan, Argenti et al. 1998; Carmelli, DeCarli et al. 1998; Pennington, Filipek et al. 2000; Eckert, Leonard et al. 2002; Geschwind, Miller et al. 2002; White, Andreasen et al. 2002) to be between 0.66 (Wright, Sham et al. 2002) and 0.97 (Pennington, Filipek et al. 2000). They agree that cortical surface area (Tramo, Loftus et al. 1995), total grey, (Pennington, Filipek et al. 2000; van Oel, Baare et al. 2001; Geschwind, Miller et al. 2002; White, Andreasen et al. 2002), and total white matter volumes (van Oel, Baare et al. 2001; White, Andreasen et al. 2002), as well as grey matter density (Thompson, Cannon et al. 2002), and individual lobe volumes (Geschwind, Miller et al. 2002; White, Andreasen et al. 2002) are all highly heritable. In the most sophisticated analysis applied to date, Thompson et al (Thompson, Cannon et al. 2002), produced a cortical map of quantified regional correlations, within MZ pairs across the brain, noting the highest regional heritabilities ofaround 90%, in a broad strip of cortex running from the frontal lobe to the parietal cortex. In contrast lateral ventricular volume exhibits the lowest heritability, and thus the greatest sensitivity to environmental effects, (van Oel, Baare et al. 2001; White, Andreasen et al. 2002; Wright, Sham et al. 2002). Gyral and sulcal architecture are also less genetically influenced with lower heritability

estimates and model fitting unable to reject a unique environmental model (Bartley, Jones et al. 1997; Eckert, Leonard et al. 2002; White, Andreasen et al. 2002). However one study (Lohmann, von Cramon et al. 1999), used a novel image analysis technique and concluded that the genetic influence varied with sulcal depth. The deepest, and ontogenetically most primitive sulci, were the most highly genetically determined.

5.5.7.1.3. Thirdly I found a significant statistical relationship between the three brain volumes and three developmental markers aligned to the genetic risk for schizophrenia, namely childhood and adolescent social development and premorbid schizotypal traits. These markers were identified in Chapter 4 as phenotypically and genotypically correlated with schizophrenia. Thus they share genetic risk with the disorder, and potentially can act as proxy markers of that genetic risk.

5.5.7.2. The conclusion that the volumetric deficits are primarily genetic in origin appears initially at odds with the only other similar study in discordant twins. Hulshoff Pol et al (Hulshoff Pol, Brans et al. 2004) reported reduced whole brain and grey matter volumes but only in twins with schizophrenia, and not the non-psychotic co-twins compared to healthy controls. They interpreted these findings as evidence of environmental, or illness specific effects. However that result was the product of an interaction test across all groups, and was not supported by a specific significant post hoc contrast between the patients and their well co-twins from discordant pairs. Furthermore the same group (Hulshoff Pol, Schnack et al. 2006), in a subsequent voxel based analysis of the same twins detected grey matter density reductions both in the patients and the unaffected relatives compared to controls. Finally, in a longitudinal 5-year follow-up MRI study of a subgroup of the same twins Brans et al (Brans, van Haren et al. 2008) reported evidence of genetically determined progressive reductions in grey matter volume both in the patients and also their unaffected co-twins, over and above changes in healthy controls.

5.5.7.3. Grey matter volume reductions have also been described in other subjects at heightened risk of schizophrenia, whether identified on clinical, familial or genetic grounds. These include people with prodromal clinical signs of schizophrenia (Pantelis, Velakoulis et al. 2003; Borgwardt, Riecher-Rossler et al. 2007), people with schizotypal personality disorder (Dickey, McCarley et al. 2001; Koo, Dickey et al. 2006; Dickey, McCarley et al. 2007), the unaffected relatives of patients (Lawrie, Whalley et al. 2001; McDonald, Grech et al. 2002; McDonald, Bullmore et al. 2004; Lawrie, McIntosh et al. 2008), also supported by meta analysis data (Boos, Aleman et al. 2007), and finally in healthy carriers of genetic polymorphisms implicated in schizophrenia (Stern, Savostyanova et al. 2008).

5.5.7.4. The white matter deficits in both the patients and their co-twins, though that did not survive multiple testing correction, is a replication of Hulshoff Pol et al's 2004 finding (Hulshoff Pol, Brans et al. 2004), interpreted as evidence of genetically determined white matter abnormalities in schizophrenia. These could underpin anatomical and functional dysconnectivity in the disorder (Stephan, Baldeweg et al. 2006; Konrad and Winterer 2008; Stephan, Friston et al. 2009). Diffusion tensor imaging (DTI) has now superseded structural imaging as the ideal method of assessing white matter integrity in psychiatric disorders (Assaf and Pasternak 2008). Unfortunately the results from a dozen and a half DTI studies in schizophrenia thus far remain inconsistent (Kanaan, Kim et al. 2005). Increasingly however they suggest that white matter tracts are disrupted in schizophrenia. This finding is replicated to certain extent in the small number of studies in unaffected relatives of patients with schizophrenia. They have reported lowered Fractional Anisotropy (an index of white matter integrity) in the inferior frontal, posterior cingulate and internal capsule, but increased FA in the anterior cingulate (Hoptman, Nierenberg et al. 2008; Munoz Maniega, Lymer et al. 2008; Camchong, Lim et al. 2009). An attempt to synthesise these potentially discrepant findings might be to suggest that white matter deficits reflect underlying non specific genetic risk to schizophrenia and psychosis (McDonald, Bullmore et al. 2004; McDonald, Bullmore et al. 2005), which underlie genetically mediated dysconnectivty as part of the fundamental vulnerability to psychosis (Whalley, Simonotto et al. 2004).

5.5.8. Specific Correlates of Schizophrenia-Unique Environmental Effects

5.5.8.1. Comparing the psychotic and non-psychotic members of MZ twins discordant for schizophrenia provides a powerful means of identifying the correlates of unique environmental factors, including factors that specifically relate to the disorder, such as treatment.

5.5.8.2. Previous MRI region of interest studies of discordant MZ twin pairs have detected disorder-specific volume deficits in frontal (Ettinger, Schmechtig et al. 2010), thalamic (Ettinger, Picchioni et al. 2007), hippocampal (Suddath, Christison et al. 1990; van Erp, Saleh et al. 2004) and lateral ventricular volumes (Baare, van Oel et al. 2001). Similarly cortical surface mapping has revealed environmentally driven within-pair differences in gray matter density between patients with schizophrenia and their unaffected MZ co-twins in the dorsolateral prefrontal cortex, superior temporal gyrus and superior parietal lobule (Cannon, Thompson et al. 2002).

5.5.8.3. My results were not consistent with these data, with no evidence, at the level of whole brain, total grey or total white matter of illness specific differences in this

cohort. This could have been due to a lack of power, and several factors in this study could come into play to increase the chance of this.

5.5.8.4. Firstly the measures selected for this analysis, whole brain volume, total grey and total white matter volumes were estimated across the entire cerebrum and lacked anatomical sensitivity. While schizophrenia is associated with widespread cortical deficits, there is evidence that the frontal and temporal lobes are most vulnerable (Shenton, Dickey et al. 2001; Goldman, Pezawas et al. 2009).

5.5.8.5. Secondly all three measures are highly heritable and, by restricting the analysis to MZ twin pairs, the high correlation of these volumes within pairs of twins, even those discordant for schizophrenia, reduced inter-individual differences that might otherwise have been detected. Indeed, in our own voxel based analysis of a sub-sample of this cohort, more subtle regionally specific deficits in the insula, superior/medial frontal, post central, cingulate and superior temporal gyri were detected(Borgwardt, Picchioni et al. 2010). Furthermore longitudinal volume changes in very similar temporal regions have been detected in individuals scanned both before and after they develop schizophrenia (Pantelis, Velakoulis et al. 2003; Job, Whalley et al. 2005; Koutsouleris, Meisenzahl et al. 2009), emphasising the specificity of these more localised deficits to schizophrenia.

5.5.8.6. There was no evidence of significant effects of antipsychotic medication in the patients, though several of these contrasts approached a trend level of significance. While antipsychotic drugs can induce cerebral structural changes (Moncrieff and Leo ; Dazzan, Morgan et al. 2005; Lieberman, Tollefson et al. 2005; Navari and Dazzan 2009; Smieskova, Fusar-Poli et al. 2009), there is emerging evidence that there may be some anatomical specificity to their site of greatest effect. First generation drugs have been associated with increases in the basal ganglia and cingulate cortex, while second generation agents have been linked to volume increases in the thalamus and temporal cortex (Chakos, Lieberman et al. 1994; Scheepers, de Wied et al. 2001; Dazzan, Morgan et al. 2005; Lieberman, Tollefson et al. 2005; Thompson, Bartzokis et al. 2009). It is possible that in this relatively small sample of patients, fifty-nine in total, prescribed a mixture of first and second generation drugs, that the study was underpowered to detect a this heterogeneous effect.

5.5.8.7. While they differed from controls, the patients did not differ whether they came from MZ discordant or concordant pairs. The two samples of patients were well matched on demographic variables and did not differ clinically. This finding does not support the second hypothesis and is at variance with our own earlier ROI studies in these twins (Ettinger, Picchioni et al. 2007; Ettinger, Schmechtig et al. 2010), in which

we detected subtle regionally specific volumetric differences between these two groups of patients, in the thalamus and parts of the frontal lobes.

5.5.9. Strengths and Limitations

5.5.9.1. In the present study I examined total, grey and white matter volumes using a whole brain approach. By surveying the whole brain in an unbiased fashion the results should provide a reliable, objective estimate of brain volume changes in schizophrenia and in those at familial risk of the disorder.

5.5.9.2. I used the 'optimized' VBM method (Good, Johnsrude et al. 2001) to minimise the potentially confounding effects of errors in stereotactic normalization (Ashburner and Friston 2000). The method is also potentially more sensitive to between-group differences (Good, Johnsrude et al. 2001).

5.5.9.3. We collected structural MR data at two centres, but using the same acquisition sequence on 'identical scanners' from the same manufacturer. This could have introduced a significant source of variance into the data, and a variety of approaches have been deployed in the past to address this (Schnack, van Haren et al. 2004; Ettinger, Picchioni et al. 2007; Ashton 2009; Moorhead, Gountouna et al. 2009). I adopted a parsimonious approach and guarded against this as a source of bias using a number of techniques. Firstly there was no systematic difference in the subjects scanned between the two sites and both members of each twin were always scanned at the same site. While there was a systematic bias between the two centres I was able to incorporate a correction factor to address this and also co-varied for scanner site in the final analyses.

5.5.9.4. Although the total sample was relatively large, the individual groups were only modest in size. As a result there remains the possibility that the study lacked statistical power. This may have particularly relevant to the comparison within discordant pairs.

5.5.9.5. As eleven of the non-psychotic co-twins in this sample met lifetime criteria for other axis I pathology, principally anxiety and affective disorders, it is possible that the volume deficits in that group could have been related to their past history of other psychiatric disorders, as opposed to their genetic risk per se. However all of them were clinically well and medication-free at the time of scanning, and the groups were otherwise well matched.

5.5.10. Conclusion

5.5.10.1. To conclude, similar volumetric abnormalities occur in patients with schizophrenia whether they come from pairs that are concordant or discordant for

schizophrenia. There was no evidence of additional differences between patients with schizophrenia and their unaffected co-twins from MZ discordant pairs, while both groups differed from healthy controls, but only if multiple testing criteria were relaxed. There was a significant association between the brain volumes and three developmental markers of the genetic risk for schizophrenia. These findings highlight the substantial familial and indeed genetic influences on these brain volume deficits in schizophrenia.

Chapter 6

6. Genetic and environmental influences on brain function in schizophrenia. An fMRI study of in the Maudsley Twin and Family cohorts.

6.1. Abstract

6.1.1. Altered neurocognition in schizophrenia could reflect the influence of both genetic and environmental factors. The aim of this study was to assess the influence of these factors on regional brain function during a task of executive function, using a case control twin-sibling study and verbal fluency as a cognitive endophenotype probe.

6.1.2. 206 subjects; 163 twins, varying in their zygosity and concordance for schizophrenia, and 43 singletons from sibling clusters, varying in their concordance for schizophrenia. The groups were matched for handedness, parental socio-economic status, and ethnicity. The groups were assessed on their behavioural performance and regional brain activation measured using functional magnetic resonance imaging, during a phonological verbal fluency task.

6.1.3. Relative to the healthy control group, patients with schizophrenia and the non-psychotic co-twins from monozygotic discordant twin pairs produced fewer correct responses. Across all groups, there was a linear trend for differential activation in fronto-temporal areas. The greater the genetic and environmental risk, the greater the activation in the left inferior frontal and left superior temporal gyri, and the greater the deactivation in the left hippocampal and middle temporal gyri bilaterally. Thus, these features were maximally evident in twins with schizophrenia, and least evident in healthy controls. When the analysis was restricted to subjects who did not have schizophrenia (and had never been treated), a similar linear trend was evident, with the non-psychotic co-twins and siblings of patients showing greater inferior frontal and left superior temporal activation, but less right parahippocampal and superior temporal activation than healthy controls. Comparison within the monozygotic twin pairs discordant for schizophrenia revealed greater activation in the medial frontal gyri and the right middle and superior frontal gyri in the affected than the non-affected twins. Full genetic modelling indicated a modest phenotypic correlation between schizophrenia and increased activity in the inferior frontal gyrus and reduced activity in the left middle temporal gyrus and left hippocampus, that was principally due to shared genetic effects.

6.1.4. Both schizophrenia and its familial vulnerability were associated with impaired performance and altered frontal, parahippocampal and temporal activation during a verbal fluency task. These were due in part, to shared genetic risk with schizophrenia.

6.2. Introduction

6.2.1. Behaviourally impaired verbal fluency performance satisfies many of the criteria for an endophenotype for schizophrenia. It is a robust feature of the disorder(Gourovitch, Goldberg et al. 1996; Vinogradov, Kirkland et al. 2003; Woodward, Ruff et al. 2003; van Beilen, Pijnenborg et al. 2004), and is evident in patients' unaffected relatives (Chen, Chen et al. 2000; Laurent, Biloa-Tang et al. 2000; Gilvarry, Russell et al. 2001; Gilvarry, Russell et al. 2001; Appels, Sitskoorn et al. 2003) and in those with schizotypal traits(Cannon, Zorrilla et al. 1994; Gilvarry, Russell et al. 2001). Moreover, twin studies show that non-psychotic twins from discordant pairs display qualitatively similar verbal fluency deficits to their ill co-twins(Goldberg, Torrey et al. 1995; Cannon, Huttunen et al. 2000), while we have found that common genetic effects link phonological verbal fluency deficits and schizophrenia(Owens, Rijsdijk et al. 2010).

6.2.2. Functional neuroimaging studies of verbal fluency in schizophrenia have reported greater activation in patients than controls in the lateral temporal cortex, with similar increases seen in patients' non-psychotic co-twins or siblings, reflecting familial effects(Spence, Liddle et al. 2000; Sommer, Ramsey et al. 2004). Findings in the prefrontal cortex are more variable(Frith, Friston et al. 1995; YurgelunTodd, Waternaux et al. 1996; Curtis, Bullmore et al. 1998; Curtis, Bullmore et al. 1999; Sommer, Ramsey et al. 2001; Sommer, Ramsey et al. 2003; Weiss, Hofer et al. 2004; Fu, Suckling et al. 2005; Spaniel, Tintera et al. 2007), with some studies describing attenuated prefrontal activation in schizophrenia(Curtis, Bullmore et al. 1998),(Andreasen, Rezai et al. 1992; Ebmeier, Blackwood et al. 1993; Gur and Gur 1995) , but others have either failed to replicate this(Frith, Friston et al. 1995; Weiss, Hofer et al. 2004; Fu, Suckling et al. 2005), or reported greater prefrontal activation in patients(Mechelli, Prata et al. 2008) and those at genetic risk(Prata, Mechelli et al. 2008) compared to controls. This inconsistency may partly reflect the potentially confounding effect of group performance differences, which have been controlled in some studies, but not in others.

6.2.3. As well as differential regional activation, several studies have also described altered functional connectivity between prefrontal and both lateral and medial temporal cortices in schizophrenia and in non-psychotic subjects at increased risk of the disorder. These studies have been a significant source of evidence to support the long-held notion that a dis-integration of regional function is fundamental to schizophrenia (Wernicke; Bleuler; Firston & Frith, 1995; McGuire & Frith 1996).

6.2.4. The aim of this study was to assess the influence of genetic, common and unique environmental influences on regional brain function in schizophrenia, using verbal fluency as a functional endophenotype probe. I compared monozygotic (MZ) and

dizygotic (DZ) twins and sibling clusters that varied in their concordance for schizophrenia.

6.2.5. In this study we wanted to establish the genetic and environmental influences on regional brain function in the context of a verbal fluency task, and then quantify the genetic relationship between regional brain function and schizophrenia. As in Chapter 4 I used a combined twin and sibling sample in the design. This offers the advantages of increasing experimental sample size, reducing sample variance and offering a better approximation of the 'true' population. In this design sibling pairs can model for dizygotic (DZ) twin pairs as they share the same degree of genetic similarity (Picchioni, Walshe et al. 2010).

6.2.6. I used functional Magnetic Resonance Imaging (fMRI) to measure regional brain function during a phonological verbal fluency task in which subjects generated words in response to letter cues. Activation in patients with schizophrenia can vary with the level of behavioural performance(Frith, Friston et al. 1995). I therefore used a paradigm that incorporated a clustered acquisition sequence that allowed us to measure task performance on-line(Amaro, Williams et al. 2002), then I restricted the analysis to images associated with correct responses, to minimize the potentially confounding effects of differences in behavioural performance.

6.2.7. My hypotheses were:

6.2.7.1. That across the entire sample, schizophrenia and its risk would be associated with altered activation in prefrontal and temporal cortices.

6.2.7.2. That qualitatively similar differences would be associated with thedegree of familial risk across non-psychotic subjects, and with the unique environmental risk that distinguishes MZ twins with schizophrenia from their non-psychotic co-twins within discordant pairs.

6.2.7.3. Finally that model fitting would detect a phenotypic correlation between schizophrenia and regional brain activity elicited by verbal fluency, and that shared genetic factors would underpin a proportion of that correlation.

6.3. Methods

6.3.1. Recruitment

6.3.1.1. The subjects participated in the Maudsley Twin(Picchioni, Walshe et al. 2010) and Family (McDonald, Marshall et al. 2006) Studies of Schizophrenia and Psychosis.

6.3.1.2. A total of 206 individuals contributed to this part of the study after Research Ethics Committee approval had been granted.

6.3.1.3. Recruitment was as described in 3.3.1.2.

6.3.1.4. Exclusion criteria applied to all groups were as in 3.3.1.3 and 4.3.1.4.

6.3.1.5. All subjects gave written informed consent after a detailed description of the study aims and method.

6.3.2. Clinical Assessment

6.3.2.1. All subjects were assessed using the methodology described in 3.3.2.1. Though to highlight current psychotic symptoms in the patients were assessed in the twins using the Scales for the Assessment of Positive (SAPS) and Negative Symptoms (SANS)(Andreasen 1983; Andreasen 1983) and for the Family Study patients using the Positive and Negative Symptom Scale (PANSS)(Kay, Opler et al. 1989). In order to compare symptomatology between the two, lifetime psychotic symptoms were then rated from all the available information with the Operational Criteria Checklist (OPCRIT)(McGuffin, Farmer et al. 1991) for all patients in the two studies with MRI data (n=153), then subject to factor analysis, yielding scores for reality distortion, negative and disorganized dimensions of psychosis. In addition if a suitable parental informant was available, all subjects were rated for premorbid adolescent shizotypal personality traits (Picchioni, Walshe et al. 2010). Furthermore intelligence was assessed by means of the WAIS-III (Wechsler Adult Intelligence Scale-III), the WAIS-R (Wechsler Adult Intelligence Scale-R), or the WASI-FSIQ-4 (Wechsler Abbreviated Scale of Intelligence). Results were converted to standardized scores (z-scores) based on the mean and standard deviation of the respective controls group.

6.3.2.2. As before in concordant twin pairs both members, and in discordant pairs one member (the proband), met DSM-IV criteria for schizophrenia or schizoaffective disorder, while their co-twin or sibling was free of any psychotic illness. Controls were free of personal or family history of psychosis or any schizophrenia spectrum disorder. Non-psychotic co-twins, siblings and controls were still included even if they met lifetime criteria for non-schizophrenia spectrum axis I pathology, and were clinically well at assessment.

6.3.3. Participants

6.3.3.1. The total sample comprised 79 twin pairs, 13 monozygotic (MZ) pairs concordant for schizophrenia, 14 MZ pairs discordant for schizophrenia in which the co-twin was free of any psychotic illness, 8 DZ pairs discordant for schizophrenia and 30 MZ and 19 DZ healthy control twin pairs (with no personal or family history of a psychotic or schizophrenia spectrum disorder) and 34 single generation sibling family clusters, 14 patients with schizophrenia, 9 of their discordant (free of any psychotic illness) siblings, and 20 healthy control siblings from unaffected families.

6.3.3.2. The probability that any of the discordant pairs would become concordant for schizophrenia in the future was low, as an average of 8.86 (SD=7.64) years in the MZ and 17.05 (10.17) years in the DZ pairs and sibling pairs had elapsed since the onset of illness in the probands(Belmaker, Pollin et al. 1974). All schizophrenia probands were clinically stable at the time of assessment, with no recent changes to their medication.

6.3.3.3. Amongst the non-schizophrenic members of the discordant twin and sibling pairs, 5 met criteria for a DSM-IV Axis I diagnosis at some point in their lives: depression and simple phobia (n=1), depression and obsessive compulsive disorder (n=1), panic disorder and depression (n=1), panic disorder, generalised anxiety disorder and depression (n=1), and generalised anxiety disorder and panic disorder (n=1). Three of the controls met diagnostic criteria for a previous DSM-IV Axis I diagnosis: depression (n=1), hypomania (n=1) and agoraphobia and drug abuse (n=1) at some point in their lifetime. None of the control twins or unaffected members of discordant pairs were unwell at the time of assessment or taking any psychotropic medication.

6.3.4. Verbal Fluency Task.

6.3.4.1. Task Description

6.3.4.1.1. We used a version of the task designed for functional MRI studies that has been described in detail previously(Fu, Morgan et al. 2001; Fu, Suckling et al. 2005). Briefly, subjects performed an overt phonological verbal fluency paradigm involving two conditions, word generation and word repetition. In the generation condition, subjects were visually presented, using Visual Basic (Microsoft Corp., Redmond, USA), with a series of cue letters (height: 7 cm, subtending a 0.5° field of view) in yellow font, on a back projected blue screen, viewed through a mirror.

6.3.4.1.2. Subjects were instructed to respond to each cue letter presentation by generating a single word that started with that letter. They were told to avoid using repetitions, grammatical variations or derivatives (e.g. run, running, runway) of an earlier response. In the word repetition condition, subjects were required to read out loud the word “REST” presented visually.

6.3.4.1.3. A blocked design was used, with the cue letters and “REST” presented in alternating blocks of seven, in an ABAB design with an inter trial interval of 4000 msec. Functional MRI data were acquired during two separate acquisition runs, each including 5 blocks of active word generation alternating with 5 blocks of baseline. This resulted in a total of 70 letter stimuli and 70 baseline trials for each subject. Verbal responses were recorded using an MRI compatible microphone onto a personal computer running Cool Edit 2000 software (Syntrillium Software Corp., [Adobe Systems Inc., San Jose, Calif.]) and later transcribed. "Incorrect" trials were collapsed

into a single error condition for the purpose of time series analysis, defined as those trials in which the subject either did not generate any response, said 'pass' or generated repetitions, derivatives or grammatical variations of an earlier response. All subjects were trained on the task before entering the MRI scanner, and reminded of the instructions once inside the scanner.

6.3.5. Behavioural Data Analysis

6.3.5.1. The percentage error responses were binomially distributed, so between group differences (MZ concordant schizophrenic, MZ and DZ/sibling discordant schizophrenic, MZ and DZ/sibling discordant non-schizophrenic, MZ and DZ and singleton control) were analysed with regression models that allowed for correlations within family clusters, and departures from normality by using the robust sandwich estimator to estimate standard errors(Binder 1983), implemented in Stata 10 (Stata Corporation, College Station, TX) as before. The significance level was set at 5%.

6.3.6. Image Acquisition.

6.3.6.1. Seventy-four T2*-weighted gradient-echo single-shot echo-planar images were acquired for each of the two four minute fifty-six second runs, on a 1.5 Tesla neuro-optimized IGE LX System (General Electric, Milwaukee) at the Maudsley Hospital, London, United Kingdom. Twelve contiguous axial planes (7mm thickness, slice skip: 0.7mm, in plane resolution = 3.75 x 3.75mm, 64x64 in plane matrix) parallel to the anterior commissure-posterior commissure line were collected in a "clustered" acquisition (TE=40 msec, flip angle=70°).

6.3.6.2. Each cue was presented visually for 750 msec and an overt verbal response could be made in the silent period of 2900 msec; an image was then acquired over 1100 msec resulting in a total repetition time (TR) of 4000 msec. This clustered or compressed acquisition sequence capitalizes on the delay of the haemodynamic response, which reaches its peak 3-5 seconds after stimulus onset[34] and allowed fMRI data to be collected after the subject had spoken thereby minimising the influence of movement artefact(Amaro, Williams et al. 2002).

6.3.6.3. A structural scan was also collected for each subject with the following acquisition parameters: TE 40 ms, TR 3 s, 43 slices, in-plane resolution 3 mm, interslice gap 0.3 mm.

6.3.7. Image Processing & Analysis.

6.3.7.1. Data were analyzed with XBAM v3.4 (Institute of Psychiatry, London, UK.) on a Sun Workstation (Sun Microsystems Inc. Santa Clara, USA).

6.3.7.2. Individual Analysis

6.3.7.2.1. The initial five images from each time series were discarded while the MR signal reached steady state and acted as the trigger for the stimuli presentation software. Images were first examined for excessive motion (> 5mm) then aligned(Bullmore, Brammer et al. 1999) to the average image over the whole time series to minimise motion related artefacts and smoothed using a Gaussian filter with a FWHM of 9mm. Responses to the experimental paradigms were detected by time-series analysis using gamma variate functions (peak responses weighted between 4 and 8 seconds) to model the blood oxygen level-dependent response.

6.3.7.2.2. The experimental condition of interest was correct word articulation, contrasted with word repetition (the baseline). In order to exclude colinearity for covariation associated with "incorrect" trials, the design model explicitly incorporated these as a separate third condition as part of the linear model in the first level analysis. First, each experimental condition was convolved separately with the 4 and 8 sec gamma functions to yield two models of the expected haemodynamic response to that condition. The weighted sum of these that gave the best fit to the time series at each voxel was then computed. In order to constrain the possible range of fits to physiologically plausible blood oxygen level-dependent responses, the constrained fitting procedure suggested by Friman et al(Friman, Borga et al. 2003) was adopted. Following this a goodness of fit statistic, the SSQ ratio (akin to a t-map), a measure of the mean power of neural response, was computed at each voxel. This was the ratio of the sum of squares of deviations from the mean intensity value due to the model (fitted time series) divided by the sum of squares due to the residuals (original time series minus model time series). The percentage BOLD signal change at each voxel was also calculated. This was ((fitmax–fitmin)/mean signal intensity)*100, where fitmax and fitmin were the maximum and minimum values of the fitted response for the time series in question.

6.3.7.2.3. In order to sample the distribution of the SSQ ratio under the null hypothesis that observed values of the SSQ ratio were not determined by the experimental design (with minimal assumptions), the time series at each voxel was permuted using a wavelet-based resampling method (Bullmore, Fadili et al. 2003; Breakspear, Brammer et al. 2004). This was repeated 20 times at each voxel and the data combined over all voxels, preserving the spatial correlational structure in the data, resulting in 20 permuted parametric maps of SSQ ratio for each subject. Combining the randomised data over all voxels yields the distribution of SSQ ratio under the null hypothesis. A test that any given voxel is activated at any required type I error can then be carried out by obtaining the appropriate critical value of SSQ ratio

from the null distribution. We have shown that this permutation method gives very good type I error control with minimal distributional assumptions for continuously and clustered acquisition sequences(Bullmore, Long et al. 2001; Breakspear, Brammer et al. 2003).

6.3.7.3. Activation during Verbal Fluency; Group Mapping

6.3.7.3.1. In order to extend inference for the condition of interest to the group level, the observed and randomised SSQ ratio maps for each subject were transformed into standard space involving first a rigid body transformation of the fMRI data into the gradient echo image of the same subject, followed by an affine transformation onto a Talairach template (Brammer, Bullmore et al. 1997). By applying the two spatial transformations for each subject to the statistical maps from the observed and wavelet-randomised data, a generic brain activation map was produced for each experimental condition. In order to allow both members of each control and concordant twin or sibling pair to contribute data to the group level analysis, and allowing for intra-pair correlations, a pair median map was first calculated for each such pair. Thus pair level maps acted as the input for the concordant and control group twin maps. The median observed SSQ ratio over all subjects at each voxel (median values were used to minimise outlier effects) was then tested at each intracerebral voxel in standard space (Talairach, Tournoux et al. 1988; Schmahmann, Doyon et al. 1999) against a critical value of the permutation distribution for median SSQ ratio, ascertained from the spatially transformed wavelet-permuted data (Brammer, Bullmore et al. 1997). In order to increase sensitivity and reduce the multiple comparison problem, hypothesis testing was carried out at the cluster level, using the method developed by Bullmore et al (Bullmore, Suckling et al. 1999), that gives excellent cluster-wise type I error control. We set the image-wise expectation for the number of false positive clusters under the null hypothesis at less than one.

6.3.7.4. Main Effect of Group (Analysis of Variance)

6.3.7.4.1.1. In order to conduct a second level analysis (ANOVA) to examine the data for a main effect of group on activation, we performed an analysis across the six experimental groups; MZ concordant for schizophrenia, MZ discordant with schizophrenia, DZ and siblings discordant with schizophrenia, MZ discordant non-psychotic, DZ and siblings discordant non-psychotic, all the healthy control subjects, with task and twin pair as repeated measures, and group categorical, using the Jonckheere-Terpstra test (J-T test) for ordered differences between classes. The value of the statistic (J) obtained from the observed data was tested against a null distribution formed by randomly permuting class labels and recalculating the statistic(Lehmann 1975). We adopted a parsimonious approach

and co-varied for age and intelligence, as these are known to influence verbal fluency performance. Analysis of variance was carried out on the effect size maps (akin to the beta-value) representing % change in BOLD response, as these were unbiased by the subject's performance, i.e. number of correct responses. This was done by first computing the difference in median effect size ratio between groups at each voxel. Subsequent inference of the probability of this difference under the null hypothesis was made by reference to the null distribution obtained by repeated random permutation of group membership and recomputation of the difference in median effect size ratios between the two groups. Cluster-level maps were then obtained, as described above, with a total number of false positive clusters for each analysis set at less than one over the entire brain. Corrections for multiple comparisons were not required, as the threshold was set on an image-wide basis.

6.3.7.5. Between Group Differences (Analysis of Variance): Post Hoc Contrasts

6.3.7.5.1. In order to more precisely identify the relationship between the experimental groups and neural activity, once an effect of group was established, specific planned post-hoc comparisons were made between respective groups. We planned the following contrasts; firstly to explore the effects of familial risk, genes and shared environment, a contrast across the three unaffected groups, MZ discordant non-psychotic, DZ and siblings discordant non-psychotic and all the healthy control subjects, was performed. Secondly in order to explore unique environmental effects we compared between the members of the MZ discordant pairs. Analysis of variance was again carried out on the effect size maps in standard space as above, co-varied for age and intelligence and cluster level maps were again set to less than one false positive cluster over the entire map.

6.3.8. Genetic Modelling

6.3.8.1. Model Fitting Analysis

6.3.8.1.1. I carried out full genetic modeling in Mx on each of the regions identified from the main effect of group analysis. I focused on those regions identified a priori, the inferior frontal gyrus, the middle and superior temporal gyrus and the medial temporal lobe.

6.3.8.2. The Twin Model

6.3.8.2.1. The twin model was as described in 4.3.5.1.

6.3.8.3. Bivariate Modelling and Polychoric Correlations

6.3.8.3.1. Bivariate modeling was as described in 4.3.5.2 but applied to partition the covariation between schizophrenia and on this occasion regional brain activity

measures, into genetic, shared and unique environmental sources of covariation. Polychoric correlations were as described in 4.3.5.4.

6.3.8.3.2. Continuous neural activity data were ordinalized into five equal classes. All correlations between schizophrenia and regional brain activity both within and across individuals, were derived from the entire sample.

6.4. Results.

6.4.1. Demographic features

6.4.1.1. There were no significant differences between the groups in handedness, ethnicity, or parental socio-economic status, but there were differences in age, IQ and the severity of psychotic symptoms at the time of scanning (Table 6.1).

6.4.2. Behavioural Data

6.4.2.1. There was a main effect of group on performance with, as anticipated, the healthy control subjects and the non-psychotic siblings and co-twins of patients making the fewest errors (Table 6.1).

6.4.3. fMRI Data

6.4.3.1. Activation within each group

6.4.3.1.1. The pattern of regional activation during word generation relative to repetition was qualitatively similar across the six groups. In MZ twins concordant for schizophrenia, activation was evident in left inferior frontal gyrus and left precentral gyrus. The MZ discordant twins with schizophrenia activated the left inferior frontal gyrus, the middle frontal gyrus bilaterally, the right striatum and the right thalamus, while the DZ discordant twins and siblings with schizophrenia showed significant activation in the left inferior/middle frontal gyrus and left insula. The MZ discordant non-psychotic twins developed activation in the same areas, and also in the lingual gyrus and cuneus bilaterally. Finally, the DZ twins and siblings discordant non-psychotic and healthy controls activated the left inferior and middle frontal gyri and left insula.

Table 6.1 Demographics of Twins & Siblings

			GROUP				Group Comparison Test
	MZ Cc Scz (n=25)	**MZ Dc Scz (n=14)**	**DZ Dc & Sibling Dc Scz (n=21)**	**MZ DC Non-psychotic (n=13)**	**DZ Dc & Sibling Dc Non-psychotic (n=17)**	**All Controls (n=116)**	
Age (years)	35.92 (10.12)	30.02 (9.56)	40.61 (12.02)	28.41 (7.72)	41.86 (14.84)	39.11 (13.43)	F[5,120]=4.07 p=0.002
N Male/Female	21/4	9/5	17/4	8/5	9/8	45/71	χ^2=18.49, df=5 p=0.002
Handedness (right/left/mixed)	19/6/0	11/3/0	20/1/0	12/1/0	13/4/0	102/9/5	χ^2=8.51, df=5 p=0.13
IQ (z-scoring)*	-2.57 (1.03)	-1.99 (1.53)	-0.87 (0.81)	-1.36 (1.24)	-0.41 (0.99)	0.01 (0.98)	F[5,114]=18.20 p<0.0001
Parental socio-economic status	2.24 (0.88)	2.14 (0.77)	2.29 (0.90)	2.15 (0.80)	2.00 (0.79)	2.58 (1.56)	F[5,119]=2.41 p=0.06
Ethnicity (% Caucasian)	96%	100%	100%	100%	100%	94.8%	F[2,120]=1.63 p=0.20
Reality distortion	-0.17 (0.83)	0.51 (0.83)	-0.08 (1.19)	NA	NA	NA	F[2,40]=2.82 p=0.07
Negative	0.02 (0.85)	0.27 (1.24)	-0.36 (0.95)	NA	NA	NA	F[2,40]=1.25 p=0.30
Disorganization	-0.55 (0.87)	0.078 (0.90)	0.47 (1.09)	NA	NA	NA	F[2,40]=2.60 p=0.09
Type of Antipsychotic (N typical/atypical/both/none)	2/10/0/3	2/8/1/3	5/14/0/0	NA	NA	NA	χ^2=0.69, df=2 p=0.71
CPZ equivalents	632.40 (441.68)	561.54 (458.78)	584.00 (874.05)	NA	NA	NA	F=[3,119]=21.9 P<0.001
Number of errors	15.44 (10.77)	15.93 (7.70)	12.05 (6.68)	17.00 (5.39)	9.82 (7.48)	10.72 (8.47)	F[5,120]=3.40 p=0.007

Legend Table 6.1 Abbreviations: Cc, concordant; Dc, discordant; MZ, monozygotic; DZ, dizygotic ; Scz schizophrenia

6.4.3.2. Main Effect of Group

6.4.3.2.1. There was a significant main effect of group, with a linear pattern of differential activation across the six groups, in specific prefrontal and temporal areas (Table 6.2).

6.4.3.2.2. In the left inferior frontal gyrus, insula and superior temporal gyrus, the groups including patients with schizophrenia showed more activation than the non-psychotic co-twins/siblings, while the latter showed greater activation than the healthy controls, (Figure 6.1, 6.2).

Table 6.2

Main Effect Across 6 Groups: Verbal Fluency vs. Baseline

Size	Tal(x)	Tal(y)	Tal(z)	Side	Cerebral Region
MZ Cc Scz ≥ MZ Dc Scz ≥ DZ Dc & Sibling Dc Scz ≥ MZ Dc Non-psychotic ≥ DZ Dc & Sibling Dc Non-psychotic ≥ Healthy Controls					
16	-40	15	-18	L	Superior Temporal Gyrus
41	-43	15	-2	L	Inferior Frontal Gyrus
45	-47	4	4	L	Precentral Gyrus
48	-43	4	15	L	Insula
MZ Cc Scz ≤ MZ Dc Scz ≤ DZ Dc & Sibling Dc Scz ≤ MZ Dc Non-psychotic ≤ DZ Dc & Sibling Dc Non-psychotic ≤ Healthy Controls					
87	-4	-56	-13	L	Cerebellum
8	51	-56	-13	R	Inferior Temporal Gyrus
68	51	-56	-2	R	Middle Temporal Gyrus
25	-29	-52	-2	L	Hippocampal Gyrus
58	32	-78	4	R	Middle Occipital Gyrus
40	-29	-59	4	L	Lingual Gyrus
15	29	-78	15	R	Precuneus
10	47	-52	15	R	Superior Temporal Gyrus
65	-25	-52	20	L	Middle Temporal Gyrus
13	0	-78	26	L	Cuneus

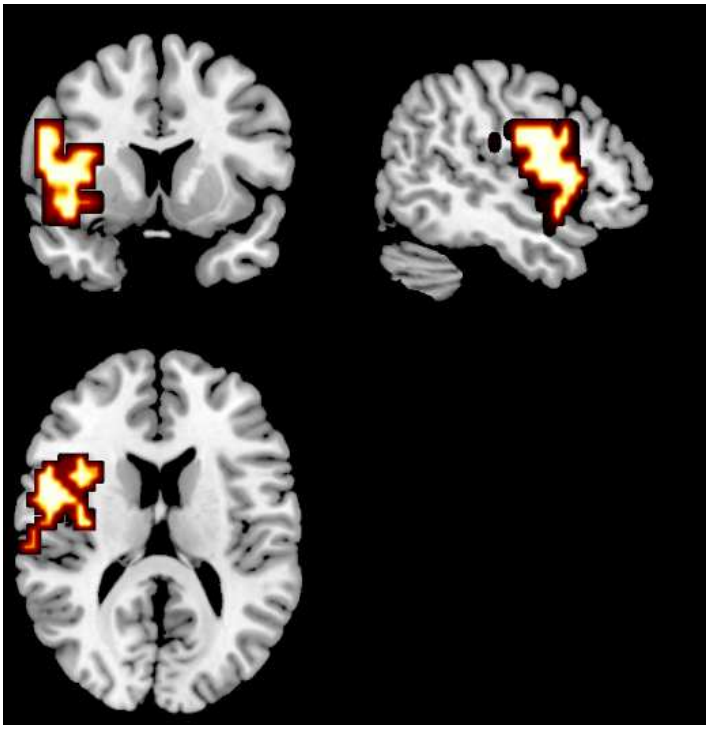
Legend Table 6.2

Abbreviations: MZ, monozygotic, DZ, dizygotic, Cc, concordant, Dc, discordant, Scz, schizophrenia, L, left, R, right. All clusters reported at voxel p=0.05 and cluster p=0.05 for the former and p=0.02 for latter contrast, yielding less than one false positive cluster respectively. Only the cluster with the largest number of voxels in each region is reported, and is limited to clusters of more than five voxels. Talairach coordinates refer to the voxel with the largest sum of squares ratio, a measure of power of neural response, in each cluster.

6.4.3.2.3. In the right superior temporal gyrus, the hippocampal/parahippocampal gyrus, and in the middle temporal gyrus and cuneus/precuneus bilaterally, this linear pattern across the groups was reversed, with controls showing the most activation,

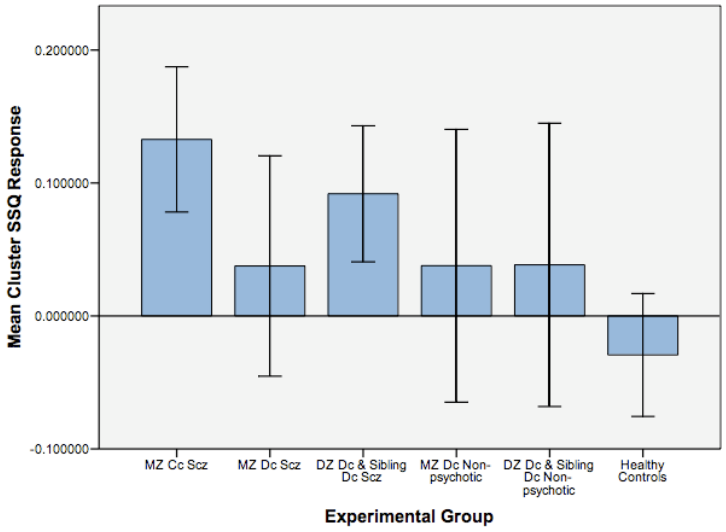
patients the least, and the non-psychotic co-twins and siblings at an intermediate level. Extracting the mean power of activation (effect size) revealed that in the left hippocampal/parahippocampal gyrus and middle temporal gyri, this reflected 'deactivation' in patients with schizophrenia, less marked deactivation in their unaffected MZ and DZ co-twins/siblings, but activation in healthy controls (Figure 6.3).

Figure 6.1



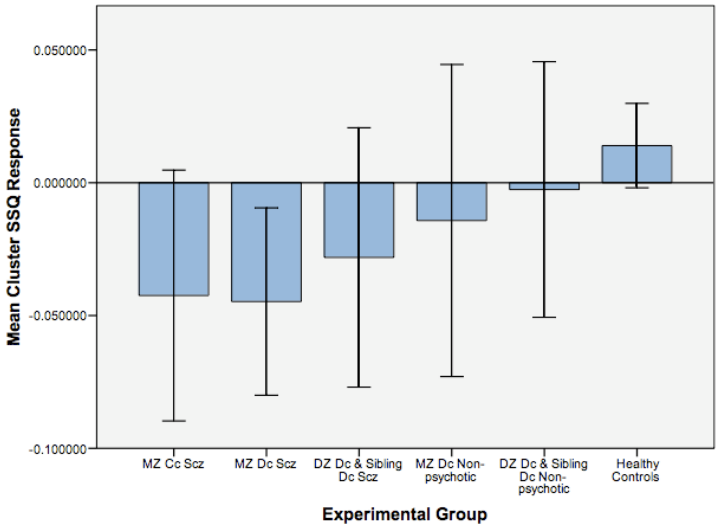
Main Effect of Group - Across 6 Groups: MZ Concordant Schizophrenia ≥ MZ Discordant Schizophrenia ≥ DZ Twin & Sibling Discordant Schizophrenia ≥ MZ Discordant Non-psychotic ≥ DZ Twin & Sibling Discordant Non-psychotic ≥ Healthy Controls. Coronal, Sagittal, Axial Views, illustrating the left inferior frontal gyrus cluster.

Figure 6.2



Extracted Signal Intensity (Mean Cluster SSQ Response) across the 6 Groups at the Left Inferior Frontal Gyrus. Coordinates: Tal(x)=-43, Tal(y)=15, Tal(z)=-2.
Abbreviations: MZ, monozygotic, DZ, dizygotic, Cc, concordant, Dc, discordant, Scz, schizophrenia.

Figure 6.3



Extracted Signal Intensity (Mean Cluster SSQ Response) across the 6 Groups at the Left Hippocampal/Parahippocampal Gyrus. Coordinates: Tal(x)=-29, Tal(y)=-52, Tal(z)=-2.
Abbreviations: MZ, monozygotic, DZ, dizygotic, Cc, concordant, Dc, discordant, Scz, schizophrenia.

6.4.3.3. Differences between Non-psychotic Groups

6.4.3.3.1. In order to identify regions specifically related to the familial (genetic plus shared environmental risk) of schizophrenia, in the absence of potentially confounding effects of illness and treatment, I restricted the above analysis to the three non-psychotic groups: non-psychotic MZ co-twins of patients, non-psychotic DZ co-twins and siblings of patients, and healthy controls.

Table 6.3 Effect Across Unaffected Subjects: Verbal Fluency vs. Baseline

Size	Tal(x)	Tal(y)	Tal(z)	Side	Cerebral Region
MZ Dc Non-psychotic ≥ DZ Dc & Sibling Dc Non-psychotic ≥ Healthy Controls					
15	-40	15	-13	L	Inferior Frontal Gyrus
30	-47	7	-2	L	Insula
41	51	26	4	R	Inferior Frontal Gyrus
5	-54	-33	4	L	Middle Temporal Gyrus
41	-43	4	9	L	Precentral Gyrus
33	54	0	9	R	Precentral Gyrus
18	-54	-22	15	L	Postcentral Gyrus
16	-58	-41	20	L	Superior Temporal Gyrus
MZ Dc Non-psychotic ≤ DZ Dc & Sibling Dc Non-psychotic ≤ Healthy Controls					
12	51	-52	-13	R	Inferior Temporal Gyrus
56	40	-44	-2	R	Hippocampal/Fusiform Gyrus
9	32	-74	-2	R	Inferior Occipital Gyrus
44	40	-41	4	R	Superior/middle Temporal Gyrus
52	32	-78	9	R	Middle Occipital Gyrus
22	25	-59	20	R	Posterior Cigulate Gyrus
18	43	-37	26	R	Inferior Parietal Lobule
6	25	-59	26	R	Precuneus

Legend Table 6.3

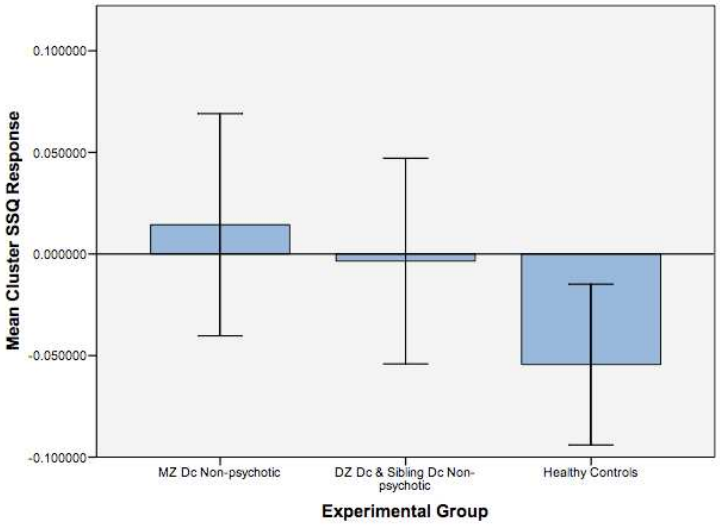
Abbreviations: MZ, monozygotic, DZ, dizygotic, Cc, concordant, Dc, discordant, Scz, schizophrenia, L, left, R, right. All clusters reported at voxel p=0.05 and cluster p=0.02, yielding less than one false positive cluster. Only the cluster with the largest number of voxels in each region is reported, and is limited to clusters with more than five voxels. Talairach coordinates refer to the voxel with the largest sum of squares ratio, a measure of power of neural response, in each cluster.

6.4.3.3.2. In the inferior frontal gyri, and the left insula and middle/superior temporal gyri, there was a linear pattern of differential activation, with greatest activation in the MZ co-twins, less activation in the DZ co-twins and siblings, and least in the healthy controls (Figure 6.4). The left inferior frontal cluster overlapped with that identified in the analysis of all six groups (above), and extended more posteriorly.

6.4.3.3.3. In the right hippocampal and middle/superior temporal gyrus, this linear pattern was reversed, with most activation in the controls, least in the MZ co-twins, and

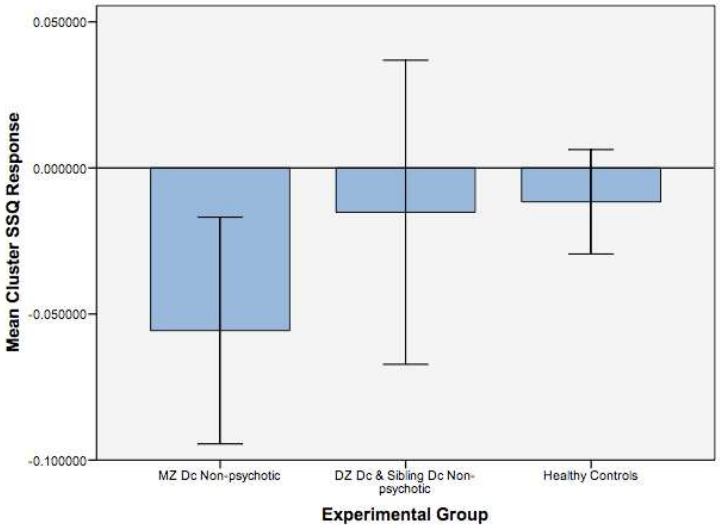
an intermediate level of activation in the DZ co-twins and siblings. Extracting the mean power of activation (effect size) from these regions revealed that this reflected relatively greater 'deactivation' with increasing familial risk (Figure 6.5).

Figure 6.4



Extracted Signal Intensity (Mean Cluster SSQ Response) across the unaffected Subjects in the Left Superior Temporal Gyrus. Coordinates: Tal(x)=-58, Tal(y)=-11, Tal(z)=4. Abbreviations: MZ, monozygotic, DZ, dizygotic, Dc, discordant.

Figure 6.5



Extracted Signal Intensity (Mean Cluster SSQ Response) across the unaffected Subjects in the Right Middle Temporal Gyrus. Coordinates: Tal(x)=29, Tal(y)=-52, Tal(z)=15. Abbreviations: MZ, monozygotic, DZ, dizygotic, Dc, discordant.

6.4.3.4. Differences Within MZ Discordant Pairs

6.4.3.4.1. The twins with schizophrenia showed greater activation than their non-psychotic co-twins in the right middle and superior frontal gyrus, and in the anterior cingulate/medial frontal gyrus bilaterally. There were no areas where the non-psychotic twins showed relatively greater activation than their co-twins with schizophrenia.

Table 6.4 MZ Discordant Schizophrenia > MZ Discordant Non-psychotic. Verbal Fluency vs. Baseline

Size	Tal(x)	Tal(y)	Tal(z)	Side	Cerebral Region
MZ Dc Scz > MZ Dc Non-psychotic					
12	0	33	20	R	Anterior Cingulate/Medial Frontal Gyrus
8	22	44	20	R	Superior Frontal Gyrus
40	32	41	26	R	Middle Frontal Gyrus
48	-4	26	31	L	Cingulate Gyrus

Legend Table 6.4

Abbreviations: MZ, monozygotic, DZ, dizygotic, L, left, R right. All clusters reported at voxel p=0.05 and cluster p=0.01, yielding less than one false positive cluster. Only the cluster with the largest number of voxels in each region is reported, and is limited to clusters with more than five voxels. Talairach coordinates refer to the voxel with the largest sum of squares ratio, a measure of power of neural response, in each cluster.

6.4.4. Genetic Modelling

6.4.4.1. On the basis of my original a priori hypotheses, I restricted the genetic modeling of signal intensity to the frontal and temporal lobe clusters identified in the main effect of group contrast described in 6.4.3.2.

6.4.4.2. Many of the cross-twin within trait confidence intervals were wide and included zero in the estimates (Table 6.5). However the majority of the cross-twin/sibling cross-trait correlations were significant. Univariate modeling indicated low and non-significant heritability h_2 (Table 6.6). The greatest were in the left hippocampus h_2= 0.40 (0.00/0.62) and the right middle temporal gyrus h_2=0.32 (0.00/0.56). However h_2 and c_2 (common environment) could not be dropped simultaneously from the model for any region without a significant decline in fit, though either could be dropped independently. This suggested a familial effect on regional activation.

6.4.4.3. Bivariate modeling (Table 6.7) revealed evidence of significant phenotypic correlations between schizophrenia and regional activation in each of the regions examined. The strongest positive correlation was in the left inferior frontal cortex (r_{ph}= 0.27), while the strongest negative correlations were in the left middle temporal gyrus (r_{ph}=-0.31) and left hippocampal gyrus (r_{ph}=-0.24). Breaking these correlations down showed that they was mainly attributable to common genetic factors, shared between each of these regions with schizophrenia (r_{pha}=0.24, r_{pha}=-0.22 and r_{pha}=-0.14, respectively). Again both the genetic and the common environmental components could be dropped alone, but not simultaneously, from the model, without a significant decline in fit.

Table 6.5. Cross-twin/sibling Within Trait and Cross-twin/sibling Cross-trait Correlations (r & 95% CI)

	Within-twin/sibling Cross-trait Correlations	*Cross-twin/sibling Within-trait Correlations*		*Cross-twin/sibling Cross-trait Correlations*	
		MZ	*DZ & Siblings*	*MZ*	*DZ & Siblings*
Left Insula	0.21 (0.07/0.33)	0.15 (-0.16/0.43)	0.61 (0.24/0.82)	0.18 (0.04/0.33)	0.37 (0.12/0.59)
Left Inferior Frontal Gyrus	0.24 (0.11/0.37)	0.14 (-0.16/0.42)	0.07 (-0.37/0.48)	0.28 (0.13/0.42)	0.04 (-0.24/0.33)
Left Superior Temporal Gyrus	0.18 (0.05/0.31)	0.07 (-0.24/0.36)	0.13 (-0.30/0.50)	0.18 (0.02/0.32)	0.22 (-0.06/0.47)
Left Precentral Gyrus	0.22 (0.09/0.34)	0.11 (-0.19/0.40)	0.35 (-0.10/0.67)	0.26 (0.12/0.40)	-0.13 (-0.39/0.16)
Right Middle Temporal Gyrus	-0.21 (-0.33/-0.08)	0.33 (0.03/0.57)	0.16 (-0.22/0.49)	-0.17 (-0.31/-0.02)	-0.07 (-0.28/0.14)
Left Middle Temporal Gyrus	-0.32 (-0.43/-0.20)	0.10 (-0.19/0.38)	0.27 (-0.11/0.58)	-0.27 (-0.40/-0.12)	-0.12 (-0.33/0.08)
Right Middle Occipital Gyrus	-0.18 (-0.31/-0.05)	0.23 (-0.07/0.49)	0.47 (0.10/0.72)	-0.22 (-0.36/-0.07)	-0.02 (-0.23/0.18)
Right Superior Temporal Gyrus	-0.07 (-0.19/0.06)	0.18 (-0.13/0.46)	0.16 (-0.23/0.51)	-0.10 (-0.24/0.06)	-0.06 (-0.26/0.16)
Left Hippocampus	-0.16 (-0.29/-0.03)	0.45 (0.18/0.65)	0.08 (-0.30/0.43)	-0.12 (-0.27/0.03)	-0.10 (-0.30/0.12)

Abbreviations: MZ, monozygotic; DZ, dizygotic

Note: The schizophrenia cross-twin correlation (SZ_{tw1}–SZ_{tw2}) is constrained to be .92 in MZ twins and .515 in DZ twins/siblings based on the point estimates of meta-analysis results, and the thresholds on the liabilities are fixed to a prevalence of 1%.

Intervals including 0 indicate non-significance.

Table 6.6. Additive genetic, common and specific environmental estimates (with 95% CI) of full ACE genetic model

	h^2	c^2	e^2
Left Insula	0.05 (0.00/0.32)	0.32 (0.02/0.53)	0.67 (0.47/0.89)
Left Inferior Frontal Gyrus	0.07 (0.00/0.42)	0.09 (0.00/0.35)	0.84 (0.58/0.97)
Left Superior Temporal Gyrus	0.02 (0.00/0.32)	0.04 (0.00/0.27)	0.94 (0.68/1.00)
Left Precentral Gyrus	0.10 (0.00/0.43)	0.17 (0.00/0.43)	0.73 (0.51/0.95)
Right Middle Temporal Gyrus	0.32 (0.00/0.56)	0.01 (0.00/0.45)	0.68 (0.44/0.95)
Left Middle Temporal Gyrus	0.06 (0.00/0.39)	0.09 (0.00/0.34)	0.85 (0.61/0.98)
Right Middle Occipital Gyrus	0.10 (0.00/0.48)	0.21 (0.00/0.46)	0.69 (0.48/0.94)
Right Superior Temporal Gyrus	0.06 (0.00/0.46)	0.12 (0.00/0.39)	0.82 (0.54/1.00)
Left Hippocampus	0.40 (0.00/0.62)	0.01 (0.00/0.45)	0.59 (0.38/0.85)

Abbreviations: MZ, monozygotic; DZ, dizygotic
Note: h^2,c^2, and e^2: heritability, shared and non-shared environmental. Confidence intervals including zero indicate non-significance.
Parameters for schizophrenia are fixed based on a prevalence of 1% and the following genetic model: h^2=.81, c^2=.11, e^2=.08

Table 6.7. The phenotypic correlations between schizophrenia and regional brain activity (r_{ph}), the decomposed sources of the correlations ($r_{ph\text{-}a}$, $r_{ph\text{-}c}$, $r_{ph\text{-}e}$) predicted by the ACE models.

	$r_{ph\text{-}a}$	$r_{ph\text{-}c}$	$r_{ph\text{-}e}$	r_{ph}
Left Insula	0.07	0.20	0.02	0.27 (0.14/0.39)
Left Inferior Frontal Gyrus	0.24	0.06	-0.03	0.27 (0.14/0.39)
Left Superior Temporal Gyrus	0.13	0.06	0.01	0.20 (0.07/0.32)
Left Precentral Gyrus	0.29	-0.02	-0.03	0.25 (0.11/0.37)
Right Middle Temporal Gyrus	-0.18	0.03	-0.04	-0.20 (-0.32/-0.07)
Left Middle Temporal Gyrus	-0.22	-0.04	-0.05	-0.31 (-0.42/-0.19)
Right Middle Occipital Gyrus	-0.28	0.08	0.03	-0.17 (-0.30/-0.04)
Right Superior Temporal Gyrus	-0.08	-0.01	0.03	-0.07 (-0.19/0.06)
Left Hippocampus	-0.09	-0.03	-0.04	-0.16 (-0.29/-0.03)

Note: r_{ph}: total phenotypic correlation; $r_{ph\text{-}a}$, $r_{ph\text{-}c}$, $r_{ph\text{-}e}$, phenotypic correlation due to additive genetic, shared environmental and specific environmental influence.
Confidence intervals including zero indicate non-significance.
Fixed genetic model for Schizophrenia used: h^2=.81, c^2=.11, e^2=.08

6.5. Discussion

6.5.1. Task Performance

6.5.1.1. There was a linear pattern of verbal fluency performance across the six groups: in general, patients made more errors than controls, with their co-twins and siblings performing at an intermediate level, in line with hypothetical variation in risk load across the groups.

6.5.1.2. This finding is consistent with previous phonological and semantic verbal fluency studies in patients(Pihlajamaki, Tanila et al. 2000; Bokat and Goldberg 2003; Vinogradov, Kirkland et al. 2003; Woodward, Ruff et al. 2003; van Beilen, Pijnenborg et al. 2004), unaffected relatives(Gourovitch, Goldberg et al. 1996; Laurent, Biloa-Tang et al. 2000; Gilvarry, Russell et al. 2001; Gilvarry, Russell et al. 2001; Vinogradov, Kirkland et al. 2003; Woodward, Ruff et al. 2003; van Beilen, Pijnenborg et al. 2004) and twins(Goldberg, Torrey et al. 1995; Cannon, Huttunen et al. 2000), which have reported performance deficits in non-psychotic relatives a level intermediate between that in patients and controls.

6.5.1.3. The only group whose performance did not fit this model comprised the MZ non-psychotic co-twins, whose performance was as impaired as in their schizophrenic co-twins and in the MZ twins concordant for schizophrenia (Table 6.1). Three of this group met lifetime criteria for affective or anxiety disorders, and their impaired performance could have been related to these disorders, as opposed to their risk for schizophrenia. However the majority of the non-psychotic co-twins had no psychiatric history, and all of them were clinically well and medication-free at the time of assessment. It is also unlikely that their poor performance reflected the presence of subjects who will subsequently develop schizophrenia, as the mean duration of discordance in these MZ twins at the time of testing was 9.25 years(Belmaker, Pollin et al. 1974).

6.5.1.4. The study was powered to detect differences at a neurophsyiological level, and genetic modelling across groups. Specific subgroups were relatively small for post hoc behavioural contrasts.

6.5.2. Functional MRI Results

6.5.2.1. Lesion and functional imaging studies of verbal fluency in healthy volunteers have consistently implicated a distributed network involving the prefrontal, cingulate, lateral and medial temporal regions engaged in this study(Damasio, Grabowski et al. 1996; Warburton, Wise et al. 1996; Phelps, Hyder et al. 1997; Schlosser, Hutchinson et al. 1998; Pihlajamaki, Tanila et al. 2000; Buchsbaum, Hickok et al. 2001; Fu, Morgan et al. 2001; Gurd, Amunts et al. 2002; Abrahams, Goldstein et al. 2003; Amunts, Weiss et al. 2004). Proficient verbal fluency performance requires the integrity of both verbal working and

long-term memory(Allen, Liddle et al. 1993; Warburton, Wise et al. 1996), and the integration of semantic knowledge and verbal search strategies, mediated by the temporal and prefrontal cortices(Gleissner and Elger 2001). Poor verbal fluency performance in schizophrenia has been attributed to impaired psychomotor speed(Woodward, Ruff et al. 2003; van Beilen, Pijnenborg et al. 2004), lexical retrieval(Elvevag, Weinstock et al. 2001; Vinogradov, Kirkland et al. 2003), memory organization(Vinogradov, Kirkland et al. 2003), word generation or recovery(Allen, Liddle et al. 1993) and compromised intelligence(Elvevag, Weinstock et al. 2001; Gilvarry, Russell et al. 2001). However the fluency deficits are more marked than those predicted by verbal intelligence alone(Crawford, Obonsawin et al. 1993; Joyce, Collinson et al. 1996) and may lie at the core of genetically transmitted cognitive effects in schizophrenia(Toulopoulou, Picchioni et al. 2007).

6.5.2.2. Verbal fluency normally engages the left inferior frontal cortex, which plays a central role in language production. Functional imaging studies have identified robust differences in activation between patients with schizophrenia and controls in this region(Curtis, Bullmore et al. 1998; Sommer, Ramsey et al. 2001; Boksman, Theberge et al. 2005; Fu, Suckling et al. 2005; Weiss, Hofer et al. 2006), with similar functional abnormalities in unaffected relatives(Spence, Liddle et al. 2000; Sommer, Ramsey et al. 2004; Whyte, Whalley et al. 2006). However, some studies have described attenuated left middle(Andreasen, Rezai et al. 1992; Ebmeier, Blackwood et al. 1993; Gur and Gur 1995) and inferior(Curtis, Bullmore et al. 1998) frontal activation in schizophrenia, while investigations that have used paced paradigms, that minimise group differences in performance, have either failed to replicate this(Frith, Friston et al. 1995; Weiss, Hofer et al. 2004; Fu, Suckling et al. 2005), or have reported greater prefrontal activation in patients(Mechelli, Prata et al. 2008) and those at genetic risk(Prata, Mechelli et al. 2008). In the present study, we used a paced paradigm, and further minimized the potential for effects of differential task performance on activation by restricting the analysis to images associated with correct responses. Consistent with other studies that have used this approach, we found that increased risk was associated with greater, rather than less prefrontal activation. Callicott has suggested that patients and their relatives require greater prefrontal activation than controls to maintain normal task performance(Callicott, Egan et al. 2003) to compensate for prefrontal ‘inefficiency’(Callicott, Mattay et al. 2003).

6.5.2.3. Relatively increased prefrontal activation in patients with schizophrenia and their unaffected relatives has also been reported in the context of other cognitive tasks (Sommer, Ramsey et al. 2001; Sommer, Ramsey et al. 2004; Weiss, Hofer et al. 2006) (Whyte, Whalley et al. 2006).

6.5.2.4. In this study, I detected evidence of a phenotypic correlation between increased

left prefrontal activation and risk for schizophrenia. Furthermore, qualitatively similar increases were present in the non-psychotic co-twins and siblings of patients, attributable to the familial risk for the disorder, and independent of potentially confounding effects of illness and treatment. Although I was underpowered to definitively discriminate between genetic and common environmental effects, there was evidence that this increase was in part driven by shared genetic factors with schizophrenia. These observations are also consistent with independent evidence from molecular genetic (as opposed to familial) studies that genetic risk for schizophrenia influences prefrontal function(Bertolino, Caforio et al. 2004; Bertolino, Caforio et al. 2006; Bertolino, Di Giorgio et al. 2008; Mechelli, Prata et al. 2008; Prata, Mechelli et al. 2008; Costafreda, Fu et al. 2009; Prata, Mechelli et al. 2009; Prata, Mechelli et al. 2009; Prata, Mechelli et al. 2009).

6.5.2.5. In contrast to the inferior frontal cortex, in the middle temporal and parahippocampal cortex, increased risk for schizophrenia was associated with relatively greater deactivation, with most deactivation in patients, less in non-psychotic relatives, and least in the controls. This is consistent with previous functional imaging studies of verbal fluency and other tasks in schizophrenia (Frith, Friston et al. 1995; Fletcher, McKenna et al. 1998), while other have not found this pattern (Spence, Liddle et al. 2000).

6.5.2.6. Reductions in medial and lateral temporal grey matter volume are among the most consistent volumetric findings in schizophrenia(Pearlson 1997; Gur, Turetsky et al. 2000; Hulshoff Pol, Schnack et al. 2001; Davatzikos, Shen et al. 2005), while qualitatively similar but less marked deficits are present in unaffected relatives(Rajarethinam, Sahni et al. 2004; Job, Whalley et al. 2005) (Honea, Meyer-Lindenberg et al. 2008). Volumetric studies in subjects with prodromal symptoms of psychosis(Borgwardt, Radue et al. 2006; Borgwardt, McGuire et al. 2007) have also detected reductions in these regions, with some evidence that reductions at presentation predict subsequent transition into psychosis. Electrophysiological studies, including data from the subjects in this(Hall, Rijsdijk et al. 2007), and another discordant twin study(Ahveninen, Jaaskelainen et al. 2006), have reported converging evidence for both genetic and illness-related abnormalities in temporal cortical processing of auditory stimuli in schizophrenia.

6.5.2.7. I detected reduced activation in the hippocampal/parahippocampal gyrus in patients with schizophrenia and their non-psychotic co-twins, findings that reflected relatively greater deactivation in this region. Genetic modelling suggested that, in the context of the verbal fluency task used here, the phenotypic correlation of hippocampal activity with schizophrenia was relatively low, -0.16, though again that the largest variance component was shared genetic risk with schizophrenia. As these differences were evident on exclusively correct trials, and our fMRI analytical method was unbiased by the number of observations, they cannot be simply attributed to impaired behavioural performance, and

are consistent with reports of reduced activation in the hippocampus in the siblings of patients with schizophrenia during a verbal working memory task(Callicott, Egan et al. 2003).

6.5.2.8. Structural neuroimaging abnormalities in the hippocampus are robust, if not unequivocal, findings in schizophrenia(Harrison and Eastwood 2001) (Gur, Turetsky et al. 2000; Wright, Rabe-Hesketh et al. 2000; Hulshoff Pol, Schnack et al. 2001; Davatzikos, Shen et al. 2005), and contemporary models of schizophrenia propose that primary pathology occurs at this site(Lodge and Grace 2007). However the findings from structural imaging studies in non-psychotic high risk groups, such as unaffected relatives or subjects with prodromal symptoms are more variable, with some reports of volume reductions (van Erp, Saleh et al. 2001; Seidman, Faraone et al. 2002; Tepest, Wang et al. 2003; Boos, Aleman et al. 2007; Honea, Meyer-Lindenberg et al. 2008) but others finding no differences(Schulze, McDonald et al. 2003; McDonald, Marshall et al. 2006; Goldman, Pezawas et al. 2008). Family and twin studies suggest that reduced hippocampal(Goldman, Pezawas et al. 2008) and temporal lobe(Brans, van Haren et al. 2008) volume in schizophrenia(Reveley, Reveley et al. 1982; Suddath, Christison et al. 1990; Baare, van Oel et al. 2001) is heritable, but influenced by environmental factors(van Erp, Saleh et al. 2004). Longitudinal MRI studies in clinically defined high risk groups also suggest that there may be a progressive loss of medial temporal volume during the transition from the high risk mental state to frank psychosis(Pantelis, Velakoulis et al. 2003; Job, Whalley et al. 2005). Our results suggest that in people with a genetic vulnerability to schizophrenia, the balance of aetiological factors between the frontal and temporal lobes and the hippocampus may vary(Heckers, Rauch et al. 1998; Meyer-Lindenberg, Poline et al. 2001).

6.5.3. Limitations

6.5.3.1. The analysis of functional MR data in this study was limited by the lack of a fully optimised analysis method to address the twin nature of the data. As has been noted in this thesis, twins by virtue of their genetic relationship violate the assumptions of independence of ANOVA. This inflates the possibility of a type I error when contrasting the controls with the other experimental groups, and increases the tendency to type II error when testing within twin pairs. I tried to guard against this through a number of steps. I matched for other potential confounders, optimised the paradigm design, produced first a pair level map for each concordant and control pair, as the input to the group level analysis, and paired the contrast between members of each twin pair.

6.5.3.2. Task performance varied significantly between the groups. I minimised the impact of this in several methodological ways. I used a clustered acquisition sequence that allowed me to measure performance while minimising head motion, using a clustered

acquisition sequence. I used a mixed blocked design that allowed me to isolate trials within each block on the basis of performance, and model out the incorrect trials. I analysed 'effect size' maps in XBAM, that are less prone to differences in observation numbers between conditions. Finally by way of reassurance, the group differences did not exclusively reflect under activity in the patients.

6.5.3.3. As the patients in the present study were the only group receiving medication, this could have contributed to between group differences, although not those between the non-psychotic co-twins/siblings and the healthy twins. We addressed this issue in our analysis that was restricted to the non-psychotic co-twins and siblings and the controls. Consistent with our prediction, the findings in both prefrontal and temporal cortex were qualitatively similar to those in the sample as a whole. This suggests that the effect of familial risk alone is qualitatively similar to that of familial plus unique environmental risk, and that the results are unlikely to be related to effects of psychotic illness or its treatment.

6.5.3.4. It is noteworthy that the contrast within MZ discordant twin pairs 6.4.3.4, yielded increased activation in the patients with schizophrenia only in the frontal and prefrontal cortex, with no differences in the temporal lobe. This is at odds with our original hypothesis that had predicted unique environmental differences in the temporal cortex. Indeed evidence from structural imaging studies, using a variety of methods, suggest that the temporal lobe is sensitive to environmental factors in schizophrenia both in lateral and medial regions. It is possible that the failure to detect this in this study is a genuine finding. Alternatively it may be a function of the choice of verbal fluency as the activation paradigm, that is more typically associated with frontal activity. It might also be a consequence of the analysis method, effectively excluding between group differences in performance.

6.5.3.5. Relative strengths of this study are that it represents only the second and certainly the largest functional imaging study of twins and unaffected siblings in schizophrenia to date. It used a robust and stable activation paradigm, chosen on the basis of off line testing to be phenotypically linked to schizophrenia and that it allowed the first genetic modelling of the link between fMRI activity and schizophrenia and thus the first quantification of fMRI activitity as an endophenotype marker for schizophrenia.

6.6. Conclusions

6.6.1. Both the familial risk for schizophrenia and the disorder itself are associated with significant functional alterations in the prefrontal, medial and lateral temporal cortices.

6.6.2. Genetic and environmental factors, aetiologically linked to schizophrenia, influenced this regional brain activity and could serve as profitable endophenotype markers for future genetic studies.

6.6.3. Future imaging studies in schizophrenia, that classify their groups on the basis of

risk genes should consider focusing on tasks that implicate these regions.

6.6.4. The extent to which these findings could reflect changes in the integration of function between these areas needs be clarified through further investigation of their anatomical and functional connectivity both in patients with schizophrenia and those at genetic risk.

Chapter 7

7. Conclusions

7.1. Summary

7.1.1. In this thesis I examined and evaluated four putative endophenotype markers for schizophrenia. I examined a twin (and family) cohort that varied in its concordance, and zygosity, for schizophrenia. I applied increasingly sophisticated assessment and analytical techniques to establish and validate the ability of these markers to meet endophenotype criteria.

7.1.2. Perhaps the least 'satisfactory' study was the structural magnetic resonance imaging dataset in Chapter 5. The sample included only MZ twins and can also be criticised for using MR data from two sites. I attempted to deal with this potential source of noise and confound in the MR data through a variety of methodological and analytical techniques. I was able to show that by adopting these techniques it is possible to collect such data reliably. While I successfully collected structural MR data on a sample of DZ discordant and DZ control twin subgroups, it was not possible to include this data in the analysis for this thesis, given the time constraints of the PhD. By virtue of the sample composition and size I was only able to apply regression models to the data analysis. These however demonstrated that the selected brain volumes were compromised in schizophrenia. They did not differ between patient groups, whether they were from MZ concordant or discordant pairs and were independent of state effects, measured as psychotic symptoms and antipsychotic treatment, at the time of scanning. The volume deficits were present in the unaffected co-twins from MZ discordant pairs more than the controls. The volumes were highly correlated within twin pairs, suggesting familial effects, but I was not able to estimate their heritability due to lack of a DZ sample. However the volume deficits were related to developmental traits, that I was able to show in Chapter 4, can act as proxy markers of the genetic risk for schizophrenia. On the basis of this evidence, the brain structural measures met endophenotype criteria on the criteria that it was possible to assess them, in this limited dataset.

7.1.3. The neurological abnormalities (Chapter 3) fulfilled all but one of the original Gottesman and Gould endophenotype criteria (Gottesman and Gould 2003). However once again I was unable to estimate their heritability due to lack of power within the sample. I was able to show that neurological abnormalities can be reliably measured at clinical examination and are associated with schizophrenia. They were correlated in families, more so in MZ than DZ pairs, strongly suggesting genetic effects, and they accumulated in the unaffected relatives of patients more so than controls. However I was unable to detect a further significant difference between the two hypothetical genetic risk groups of unaffected co-twins. While the neurological abnormalities were not influenced by the 'state' of

schizophrenia, in terms of psychotic symptoms, there was evidence that they were sensitive to, or could be influenced by antipsychotic medication. On the basis of this evidence the neurologiocal abnormalities met many endophenotype criteria, though their demonstrable sensitivity to antipsychotic treatment may limit their usefulness to some extent.

7.1.4. The regression and genetic modelling analyses applied to the child and adolescent developmental traits (Chapter 4) and neural activity associated with verbal fluency (Chapter 6) allowed a more detailed analysis of their respective endophenotype credentials. The results showed that the developmental markers were associated with schizophrenia and were also elevated in the unaffected family members in a manner that strongly suggested genetic effects. The modelling analysis was able to quantify both their heritability and their phenotypic correlation with schizophrenia, and finally estimate that additive genetic effects were responsible for that phenotypic relationship due to shared genetic risk.

7.1.5. Turning to verbal fluency performance and its associated neural activity, this was also compromised in schizophrenia and segregated in families, following the hypothetical pattern of genetic risk. The cross twin-within trait and heritability estimates were however non significant. However I was able to show a significant phenotypic correlation between schizophrenia and increased neural activity in the inferior frontal gyrus and reduced activity in the left middle temporal gyrus and left hippocampus. These phenotypic correlations were principally due to shared genetic effects with schizophrenia.

7.2. On balance the evidence in this thesis is most conclusive for the developmental and verbal fluency markers, supporting their role as endophenotype markers. However, in comparative terms, this may relate more to sample size and methodological factors, than their own inherent properties, compared to the other candidates. Given their selection on the basis of past work, all of the markers chosen for inclusion in this thesis have demonstrated that they meet many endophenotype criteria.

7.3. Critical Analysis of the Endophenotype Concept and Suggestions for Future Directions

7.3.1. Endophenotypes have risen to prominence in mental illness research because of our poor grasp of the aetiological and pathophysiological complexity of these disorders and as a consequence our inability to define the margins of these disorders more distinctly. Endophenotypes have evolved conceptually as quanitative traits, sited on the aetiological pathway between genotype and phenotype, and closer to the genetic effects, they offer a lure of relative simplicity and precision.

7.3.2. Endophenotypes are not equivalent to biomarkers. A biomarker is an indicator of a normal or pathological processes (Atkinson, Colburn et al. 2001). Biomarkers are disease specific and index the presence or severity of their disorder (Ritsner and Gottesman 2009). By way of contrast endophenotypes are quantifiable biological variants that are stable trait markers of the genetic vulnerability for a disorder (Ritsner and Gottesman 2009) and independent of its severity.

7.3.3. It was highlighted in 2.3 that there is no unanimity as to how endophenotypes should be characterised. The two most influential definitions are that an endophenotype should be (Gottesman and Gould 2003):

- associated with the illness in the population
- state independent
- heritable
- co-segregate in families
- present in unaffected members more than the general population.

7.3.4. Influenced by these identifiable criteria Preston and Weinberger then suggested that an endophenotype should be (Preston and Weinberger 2005):

- a quantitative biological trait that is reliable and reasonably heritable
- associated with variant alleles that distinguish patients and their unaffected siblings from healthy controls on quantitative measures
- reflect a less complex genetic architecture than the disorder

7.3.5. Combining these criteria engenders a series of assumptions about endophenotypes that underpins their potential usefulness:

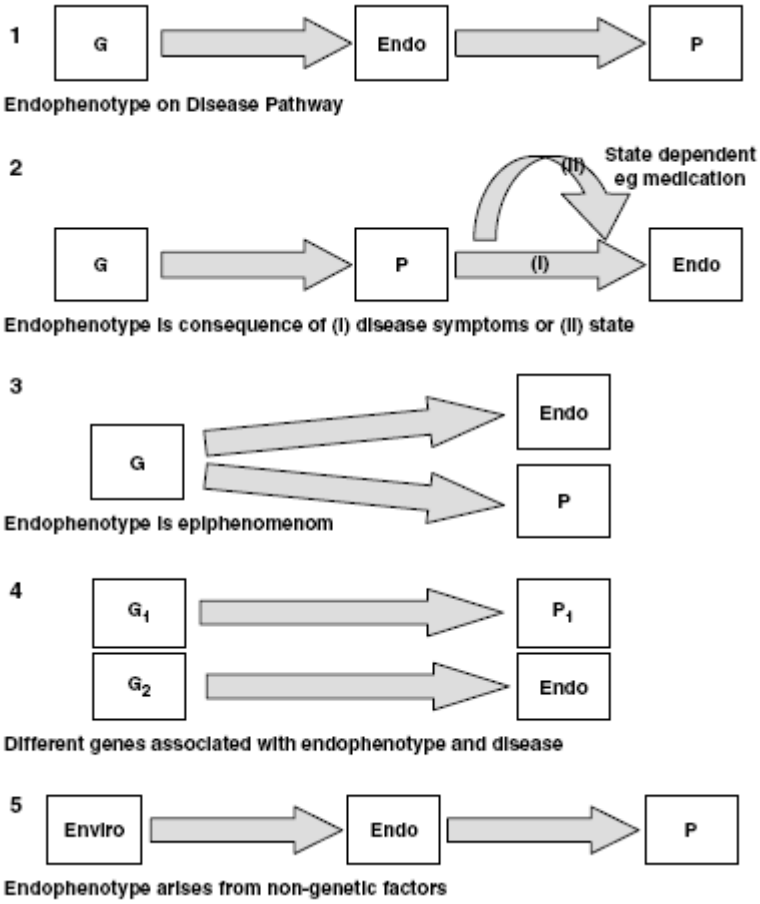
- that they are genetically less complex than the exophenotype
- that effect sizes for their genetic effects are larger
- that they lie on the pathophysiological pathway from genotype to phenotype
- that they are specific to a disorder.

7.4. However, as some have pointed out, none of these assumptions can necessarily be shown to be true on the basis of the majority of the published literature (Walters and Craddock 2007).

7.4.1. The principle role of endophenotypes is to support gene discovery and while it is not part of the original definition, it is accepted that endophenotypes ideally should be, genetically less complex than the original exophenotype. However there is little evidence to support this conclusion (Flint and Munafo 2007; Walters and Craddock 2007; Prasad and Keshavan 2008). Firstly the vast majority of studies that have attempted to identify either directly or indirectly the endophenotypic credentials of candidate markers, have been unable to establish their heritability. There is even less evidence to establish their position on the aetiological pathway between genes and the disorder, though this point is often inferred from

their detection in unaffected relatives and their state independence in patients. However only longitudinal study designs can truly establish this type of causal relationship. Furthermore what studies have so for been conducted that assess the genetic influences on putative endophenotypes have produced mixed results (Paunio, Tuulio-Henriksson et al. 2004; Dick, Jones et al. 2006; Flint and Munafo 2007). Indeed the inability of many earlier studies to definitively address these questions has lead to the conclusion that many of these markers may in fact be epiphenomena of schizophrenia.

Fig 7.1

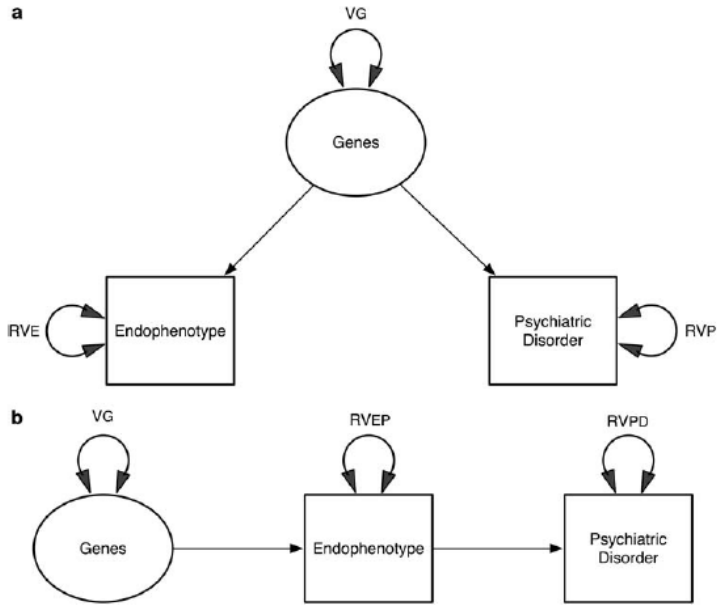


This shows in simplified, schematic terms some of the possible relationships between putative endophenotypes, gene and disease. In reality, different combinations of these simplified scenarios are likely. G, genes; Enviro, environmental factors; Endo, putative endophenotype; P, disease phenotype.

Reprinted by permission from Macmillan Pulishers Ltd: Molecular Psychiatry. JTR Walters and MJ Craddock. Endophenotypes in psychiatric genetics (2007). Molecular Psychiatry 12; 886-890.

7.4.2. Figure 7.1 illustrates some of the possible aetiological models that could link genes, an endophenotype and a psychiatric disorder. Model 1 is of a 'true' endophenotype, and illustrates a mediational model that links genes, the endopheotype and the disorder. It is the model that we usually associate with the endophenotype concept. Model 3 is a risk-liability model and will successfully meet all of Gottesman and Goulds' endophenotype characteristics, but is an epiphenomenon, not an endophenotype. It will share genetic risk, but not be causally linked to the illness. The two pathways are contrasted in Fig 7.2. Both will allow genes to cause the 'endophentype' and the illness, both will appear to be heritable, both will lead to elevated rates of the endophenotype in patients' unaffected relatives. However only the mediational model in pathway (b) predicts that the genetically determined pathology that drives the endophenotype is then causally related to the psychiatric illness. From a clinical perspective it also vitally predicts that a successful intervention against the endophenotype, will have a positive impact of the illness. Again only longitudinal study designs can address this question satisfactorily and by their very nature the developmental markers assessed in Chapter 4, especially in the context of the current expansion of First Episode Psychosis and At Risk Mental State services currently, hint at a possible opportunity to make just such an intervention for the benefit of patients.

Figure 7.2



(c) A liability-index model for endophenotypes (EPs). Genetic variance VG influences both the EP and psychiatric disorder (PD). These observed variables also have residual variation, RVEP and RVPD, due to other sources.

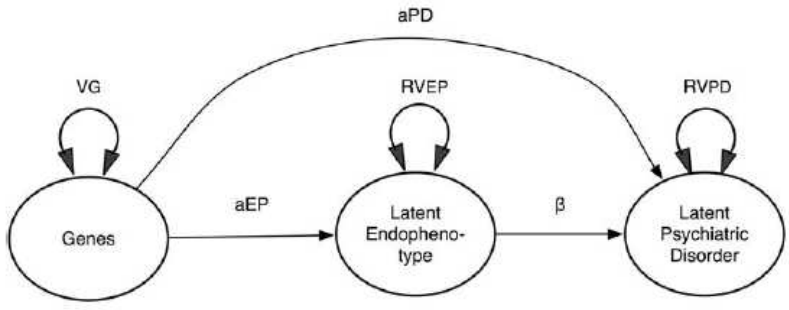
(d) A mediational model for EPs. Genetic variance causes variation in the EP, which in turn causes variation in PD. EP and PD have residual variance components RVEP and RVPD, respectively.

Reprinted by permission from Macmillan Publishers Ltd: Molecular Psychiatry. KS Kendler and MC Neale. Endophenotype: a conceptual analysis (2010). Molecular Psychiatry 15; 789-797.

7.4.3. A further criticism of endophenotype models is that while they assume that causal relationships are one way, this need not be the case. For instance it may be that the presence of neurological abnormalities, or impaired social ability increase the risk for schizophrenia, but equally it could be that developing schizophrenia increases the risk of neurological abnormalities and impaired social opportunity. Once again future prospective longitudinal studies are needed to address this issue.

7.4.4. There is little or no evidence that all of the genetic risk for the illness needs to pass through the endophenotype. Indeed it is likely that some of the genetic effects will by-pass the endophenotype, directly acting on the illness (Fig 7.3). Furthermore there may be genes that act on the illness alone, while others act on the endophenotype alone. The modelling studies I conducted in this thesis are one of the only analytical models that can quantify shared genetic risk and address this problem through estimation of the r_g, the genetic correlation. Future studies should estimate this for multiple endophenotypes in combination, to produce composite or 'extended endophenotypes' with the greatest r_g, for future genetic studies (See 7.3.10).

Figure 7.3



A nonexclusive mediational model for endophenotypes (EPs) including a direct causal path from genes to the psychiatric disorder (aPD) that does not pass through EP.

Reprinted by permission from Macmillan Pulishers Ltd: Molecular Psychiatry. KS Kendler and MC Neale. Endophenotype: a conceptual analysis (2010). Molecular Psychiatry 15; 789-797.

7.4.5. The vast majority of research activity in relation to endophenotpyes in the last fifteen years has focussed on their relationship with genes and genetic risk, while either ignoring the environment, or viewing the environment as a confound. This has in part reflected the prevailing focus of academic interest but perhaps also reflected a lack of confidence in the tools and instruments to measure the environment. The role and importance of the environment in schizophrenia, while perhaps neglected has never gone away. More recently interest in the role of the environment and genetics and the interactions and correlations between the two has grown. Furthermore while the environment's influence on an endophenotype in the past might have been seen as a nuisance, it now seems clear that endophenotypes can perform exactly the same role but as indices of the environmental impact on an exophenotype, just as they do for genes. In line with this change in thinking and in response to a need to develop more sophisticated aetiological models to discover the risk variants that underpin schizophrenia future genetic studies of endophenotypes should incorporate measures both of environmental adversity and support. This consideration will become increasingly important if we are to develop a better understanding of the link between peoples' lives, at the individual and wider societal levels, and their risk of psychosis, and the mechanisms that mediate that risk. Furthermore through a better understanding of the effects of the environment and by identifying those people at greatest genetic risk to that environmental adversity, and who might benefit most from support, that targeted prophylactic strategies could be developed and deployed in the most efficient and effective manner possible.

7.4.6. One further research strategy to address the specific concerns around endophenotypes might be to combine multiple endophenotype measures in the population being studied, a concept some have called 'extended endophenotypes' (Prasad and Keshavan 2008), though perhaps composite is more accurate. Criteria already proposed for this concept include that:

- each candidate trait should independently meet endophenotype criteria
- the extended endophenotype should involve at least two levels of markers, e.g. physiological and structural
- those selected should be correlated within individuals and co-segregate in families

- those selected should be mechanistically related (given our current knowledge).

7.4.7. However additional criteria for such an extended endophenotype model should be that each of the candidate markers is not only linked to schizophrenia, but that this link is mediated by genes (Walters and Craddock 2007) and that the link is shared between the markers themselves. Fundamentally this means that they should be phenotypically and genotypically correlated both with schizophrenia and each other. Subjects in future will then be selected and stratified for genetic linkage and association studies on the basis of their status for the extended endophenotype marker.

7.4.8. By using such strategies our ability to detect the genes responsible for schizophrenia, and thereby to understand the pathology of this disorder will improve. Through that route we will develop better, safer, more effective individualised treatments and so improve the prognosis for our patients.

References:

(1993). The International Statistical Classification of Diseases and Related Health Problems Tenth Revision. Geneva, World Health Organisation.

Abrahams, S., L. H. Goldstein, et al. (2003). "Functional magnetic resonance imaging of verbal fluency and confrontation naming using compressed image acquisition to permit overt responses." Human Brain Mapping **20**(1): 29-40.

Addington, J. and D. Addington (2005). "Patterns of premorbid functioning in first episode psychosis: relationship to 2-year outcome." Acta Psychiatrica Scandinavica **112**(1): 40-46.

Ahveninen, J., I. P. Jaaskelainen, et al. (2006). "Inherited auditory-cortical dysfunction in twin pairs discordant for schizophrenia." Biological Psychiatry **60**(6): 612-620.

Allan, C. L., A. G. Cardno, et al. (2009). "Twin study of illness history variables in psychosis." Schizophrenia Research **115**(2-3): 237-244.

Allen, H. A., P. F. Liddle, et al. (1993). "Negative features, retrieval-processes and verbal fluency in schizophrenia." British Journal of Psychiatry **163**: 769-775.

Allen, M. G. and W. Pollin (1970). "SCHIZOPHRENIA IN TWINS AND DIFFUSE EGO BOUNDARY HYPOTHESIS." American Journal of Psychiatry **127**(4): 437-&.

Amaro, E., S. C. R. Williams, et al. (2002). "Acoustic noise and functional magnetic resonance imaging: Current strategies and future prospects." Journal of Magnetic Resonance Imaging **16**(5): 497-510.

American Psychiatric Association (1994). Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition. Washington, DC, American Psychiatric Association.

Amunts, K., P. H. Weiss, et al. (2004). "Analysis of neural mechanisms underlying verbal fluency in cytoarchitectoncially defined stereotaxic space-The roles of Brodmann areas 44 and 45." Neuroimage **22**: 42-56.

Andreasen, N. C. (1983). The Scale for the Assessment of Negative Symptoms (SANS). Iowa City, IA., Iowa University.

Andreasen, N. C. (1983). The Scale for the Assessment of Positive Symptoms (SAPS). Iowa City, IA., Iowa University.

Andreasen, N. C., K. Rezai, et al. (1992). "Hypofrontality in neuroleptic-naive patients and in patients with chronic schizophrenia. Assessment with xenon 133 single-photon emission computed tomogrpahy and the Tower of London." Arch Gen Psychiatry **49**: 943-958.

Annett, M. (1970). "A classification of hand preference by association analysis." Br J Psychol **61**(3): 303-321.

Appels, M. C. M., M. M. Sitskoorn, et al. (2003). "Cognitive dysfunctions in parents of schizophrenic patients parallel the deficits found in patients." Schizophrenia Research **63**(3): 285-293.

Argyropoulos, S. V., S. Landau, et al. (2008). "Twins discordant for schizophrenia: psychopathology of the non-schizophrenic co-twins." Acta Psychiatrica Scandinavica **118**(3): 214-219.

Arieti, S. (1949). "An interpretation of the divergent outcome of schizophrenia in identical twins. ." Psychiatric Quarterly **18**: 587-599.

Arnone, D., J. Cavanagh, et al. (2009). "Magnetic resonance imaging studies in bipolar disorder and schizophrenia: meta-analysis." British Journal of Psychiatry **195**(3): 194-201.

Arseneault, L., M. Cannon, et al. (2002). "Cannabis use in adolescence and risk for adult psychosis: longitudinal prospective study." British Medical Journal **325**(7374): 1212-1213.
Ashburner, J. and K. J. Friston (2000). "Voxel-based morphometry - The methods." NeuroImage **11**(6): 805-821.
Ashton, E. (2009). "Quantitative MR in Multi-center clinical trials." Journal of Magenetic Resonance Imaging **31**: 279-288.
Assaf, Y. and O. Pasternak (2008). "Diffusion tensor imaging (DTI)-based white matter mapping in brain research: A review." Journal of Molecular Neuroscience **34**(1): 51-61.
Atkinson, A. J., W. A. Colburn, et al. (2001). "Biomarkers and surrogate endpoints: Preferred definitions and conceptual framework." Clinical Pharmacology & Therapeutics **69**(3): 89-95.
Baare, W. F. C., C. J. van Oel, et al. (2001). "Volumes of brain structures in twins discordant for schizophrenia." Archives of General Psychiatry **58**(1): 33-40.
Baca-Garcia, E., M. M. Perez-Rodriguez, et al. (2007). "Diagnostic stability of psychiatric disorders in clinical practice." British Journal of Psychiatry **190**: 210-216.
Bachman, P., J. Kim, et al. (2003). "EEG synchronization and spatial working memory in schizophrenia patients, their unaffected co-twins, and controls." Psychophysiology **40**: S24-S24.
Bachman, P., J. Kim, et al. (2008). "Abnormally high EEG alpha synchrony during working memory maintenance in twins discordant for schizophrenia." Schizophrenia Research **103**(1-3): 293-297.
Bailer, J., W. Brauer, et al. (1996). "Premorbid adjustment as predictor of outcome in schizophrenia: Results of a prospective study." Acta Psychiatrica Scandinavica **93**(5): 368-377.
Baron, M., R. Gruen, et al. (1985). "A family study of schizophrenic and normal control probands: implications for the spectrum concept of schizophrenia." American Journal of Psychiatry **142**(4): 447-455.
Bartley, A. J., D. W. Jones, et al. (1997). "Genetic variability of human brain size and cortical gyral patterns." Brain **120**: 257-269.
Battaglia, M., L. Bernardeschi, et al. (1995). "A family study of schizotypal disorder." Schizophrenia Bulletin **21**(1): 33-45.
Bazire, S. (2007). Psychotropic Drug Directory. Aberdeen, HealthComm UK Limited
Belmaker, R., W. Pollin, et al. (1974). "Follow-up of Monozygotic Twins Discordant for Schizophrenia." Archives of General Psychiatry **30**(2): 219-222.
Bentall, R. P., G. S. Claridge, et al. (1988). "The multidimensional nature of schizotypal traits: A factor analytic study with normal subjects." British Journal of Clinical Psychology **28**: 363-375.
Bergman, A. J., J. M. Silverman, et al. (2000). "Schizotypal symptoms in the relatives of schizophrenia patients: An empirical analysis of the factor structure." Schizophrenia Bulletin **26**(3): 577-586.
Berman, K. F., E. F. Torrey, et al. (1992). "REGIONAL CEREBRAL BLOOD-FLOW IN MONOZYGOTIC TWINS DISCORDANT AND CONCORDANT FOR SCHIZOPHRENIA." Archives of General Psychiatry **49**(12): 927-934.
Bertolino, A., G. Caforio, et al. (2004). "Interaction of COMT Val(108/158) met genotype and olanzapine treatment on prefrontal cortical function in patients with schizophrenia." American Journal of Psychiatry **161**(10): 1798-1805.

Bertolino, A., G. Caforio, et al. (2006). "Prefrontal dysfunction in schizophrenia controlling for COMT Val(158)Met genotype and working memory performance." Psychiatry Research-Neuroimaging **147**(2-3): 221-226.
Bertolino, A., A. Di Giorgio, et al. (2008). "Epistasis between dopamine regulating genes identifies a nonlinear response of the human hippocampus during memory tasks." Biological Psychiatry **64**(3): 226-234.
Bezchlibnyk-Butler, K. Z., J. J. Jeffries, et al. (1994). "Clinical handbook of psychotropic drugs, Fourth edition." Clinical handbook of psychotropic drugs, Fourth edition: ii+129p.
Binder, D. A. (1983). "On the Variances of Asymptotically Normal Estimators from Complex Surveys." International Statistical Review **51**(3): 279-292.
Bland, J. M. and D. G. Altman (1986). "Statistical-methods for assessing agreement between methods of clinical measurement." Lancet **1**(8476): 307-310.
Bleuler, E. (1911). Dementia Praecox oder die Gruppe der Schizophrenien. Liepzig, Deuticke.
Bokat, C. E. and T. E. Goldberg (2003). "Letter and category fluency in schizophrenic patients: a meta-analysis." Schizophrenia Research **64**(1): 73-78.
Boklage, C. E. (1977). "SCHIZOPHRENIA, BRAIN ASYMMETRY DEVELOPMENT, AND TWINNING - CELLULAR RELATIONSHIP WITH ETIOLOGICAL AND POSSIBLY PROGNOSTIC IMPLICATIONS." Biological Psychiatry **12**(1): 19-35.
Boksman, K., J. Theberge, et al. (2005). "A 4.0-T fMRI study of brain connectivity during word fluency in first-episode schizophrenia." Schizophrenia Research **75**: 247-263.
Bonan, I., A. M. Argenti, et al. (1998). "Magnetic resonance imaging of cerebral central sulci: A study of monozygotic twins." Acta Geneticae Medicae et Gemellologiae **47**(2): 89-100.
Boomsma, D., A. Busjahn, et al. (2002). "Classical twin studies and beyond." Nature Review Genetics **3**(11): 872-882.
Boos, H. B., A. Aleman, et al. (2007). "Brain volumes in relatives of patients with schizophrenia." Archives of General Psychiatry **64**: 297-304.
Boos, H. B. M., A. Aleman, et al. (2007). "Brain volumes in relatives of patients with schizophrenia - A meta-analysis." Archives of General Psychiatry **64**(3): 297-304.
Borgwardt, S. J., P. K. McGuire, et al. (2007). "Structural brain abnormalities in individuals with an at-risk mental state who later develop psychosis." British Journal of Psychiatry **191**: S69-S75.
Borgwardt, S. J., M. M. Picchioni, et al. (2010). "Regional Gray Matter Volume in Monozygotic Twins Concordant and Discordant for Schizophrenia." Biological Psychiatry **67**(10): 956-964.
Borgwardt, S. J., M. M. Picchioni, et al. (In Press). "Regional Gray Matter Volume in Monozygotic Twins COncordant and Discordant for Schizophrenia." Biological Psychiatry.
Borgwardt, S. J., E. W. Radue, et al. (2006). "Radiological findings in individuals at high risk of psychosis." Journal of Neurology Neurosurgery and Psychiatry **77**(2): 229-233.
Borgwardt, S. J., A. Riecher-Rossler, et al. (2007). "Regional gray matter volume abnormalities in the at risk mental state." Biological Psychiatry **61**(10): 1148-1156.

Boydell, J., J. van Os, et al. (2004). "The association of inequality with the incidence of schizophrenia - An ecological study." Social Psychiatry and Psychiatric Epidemiology **39**(8): 597-599.

Bracha, H. S., E. F. Torrey, et al. (1991). "SUBTLE SIGNS OF PRENATAL MALDEVELOPMENT OF THE HAND ECTODERM IN SCHIZOPHRENIA - A PRELIMINARY MONOZYGOTIC TWIN STUDY." Biological Psychiatry **30**(7): 719-725.

Bracha, H. S., E. F. Torrey, et al. (1992). "2ND-TRIMESTER MARKERS OF FETAL SIZE IN SCHIZOPHRENIA - A STUDY OF MONOZYGOTIC TWINS." American Journal of Psychiatry **149**(10): 1355-1361.

Brammer, M. J., E. T. Bullmore, et al. (1997). "Generic brain activation mapping in functional magnetic resonance imaging: A nonparametric approach." Magnetic Resonance Imaging **15**(7): 763-770.

Brans, R., N. Van Haren, et al. (2007). "Structural brain changes in twin pairs discordant for schizophrenia: A 5-year follow-up MRI study." Schizophrenia Bulletin **33**(2): 329-330.

Brans, R. G. H., N. E. M. van Haren, et al. (2008). "Heritability of changes in brain volume over time in twin pairs discordant for schizophrenia." Arch Gen Psychiatry **65**(11): 1259-1268.

Brans, R. G. H., N. E. M. van Haren, et al. (2008). "Heritability of Changes in Brain Volume Over Time in Twin Pairs Discordant for Schizophrenia." Archives of General Psychiatry **65**(11): 1259-1268.

Bray, N. J., A. Preece, et al. (2005). "Haplotypes at the dystrobrevin binding protein 1 (DTNBP1) gene locus mediate risk for schizophrenia through reduced DTNBP1 expression." Human Molecular Genetics **14**(14): 1947-1954.

Breakspear, M., M. Brammer, et al. (2003). "Construction of multivariate surrogate sets from nonlinear data using the wavelet transform." Physica D-Nonlinear Phenomena **182**(1-2): 1-22.

Breakspear, M., M. J. Brammer, et al. (2004). "Spatiotemporal wavelet resampling for functional neuroimaging data." Human Brain Mapping **23**(1): 1-25.

Bressers, W. M. A., A. W. Eriksson, et al. (1987). "Increasing tren in monozygotic twinning rate." Acta Geneticae Medicae Et Gemellologiae **36**(3): 397-408.

Bridle, N., C. Pantelis, et al. (2002). "Thalamic and caudate volumes in monozygotic twins discordant for schizophrenia." Australian and New Zealand Journal of Psychiatry **36**(3): 347-354.

Broome, M. R., J. B. Wooley, et al. (2005). "What causes the onset of psychosis?" Schizophrenia Research **79**: 23-34.

Buchanan, R. W. and D. W. Heinrichs (1989). "The Neurological Evaluation Scale (Nes) - a Structured Instrument for the Assessment of Neurological Signs in Schizophrenia." Psychiatry Research **27**(3): 335-350.

Buchsbaum, B. R., G. Hickok, et al. (2001). "Role of left posterior superior temporal gyrus in phonological processing for speech perception and production." Cognitive Science **25**(5): 663-678.

Bullmore, E., J. Fadili, et al. (2003). "Wavelets and statistical analysis of functional magnetic resonance images of the human brain." Statistical Methods in Medical Research **12**(5): 375-399.

Bullmore, E. T., M. J. Brammer, et al. (1999). "Methods for diagnosis and treatment of stimulus-correlated motion in generic brain activation studies using fMRI." Human Brain Mapping **7**(1): 38-48.

Bullmore, E. T., C. Long, et al. (2001). "Coloured noise and computational inference in neurophysiological (fMRI) time series analysis: Resampling methods in time and wavelet domains." Human Brain Mapping **12**: 61-78.
Bullmore, E. T., J. Suckling, et al. (1999). "Global, voxel, and cluster tests, by theory and permutation, for a difference between two groups of structural MR images of the brain." Ieee Transactions on Medical Imaging **18**(1): 32-42.
Callicott, J. H., M. F. Egan, et al. (2003). "Abnormal fMRI response of the dorsolateral prefrontal cortex in cognitively intact siblings of patients with schizophrenia." American Journal of Psychiatry **160**(4): 709-719.
Callicott, J. H., V. S. Mattay, et al. (2003). "Complexity od prefrontal cortical dysfunction in schizophrenia: more than up and down." American Journal of Psychiatry **160**(12): 2209-2215.
Camchong, J., K. O. Lim, et al. (2009). "Frontal white matter integrity as an endophenotype for schizophrenia: diffusion tensor imaging in monozygotic twins and patients' nonpsychotic relatives." Frontiers in Human Neuroscience **3**.
Cannon-Spoor, H. E., S. G. Potkin, et al. (1982). "Measurement of Premorbid Adjustment in Chronic-Schizophrenia." Schizophrenia Bulletin **8**(3): 470-484.
Cannon, M., A. Caspi, et al. (2002). "Evidence for early-childhood, pan-developmental impairment specific to schizophreniform disorder - Results from a longitudinal birth cohort." Archives of General Psychiatry **59**(5): 449-456.
Cannon, M., P. Jones, et al. (1997). "Premorbid social functioning in schizophrenia and bipolar disorder: Similarity and differences." American Journal of Psychiatry **154**(11): 1544-1550.
Cannon, T. D., M. O. Huttunen, et al. (2000). "The inheritance of neuropsychological dysfunction in twins discordant for schizophrenia." American Journal of Human Genetics **67**(2): 369-382.
Cannon, T. D., J. Kaprio, et al. (1998). "The genetic epidemiology of schizophrenia in a Finnish twin cohort - A population-based modeling study." Archives of General Psychiatry **55**(1): 67-74.
Cannon, T. D. and M. C. Keller (2006). "Endophenotypes in the genetic analysis of mental disorders." Annual Review of Clinical Psychology **2**: 267-290.
Cannon, T. D., P. M. Thompson, et al. (2002). "Cortex mapping reveals regionally specific patterns of genetic and disease-specific gray-matter deficits in twins discordant for schizophrenia." Proceedings of the National Academy of Sciences of the United States of America **99**(5): 3228-3233.
Cannon, T. D., T. G. M. van Erp, et al. (1998). "Regional gray matter, white matter, and cerebrospinal fluid distributions in schizophrenic patients, their siblings, and controls." Archives of General Psychiatry **55**(12): 1084-1091.
Cannon, T. D., L. E. Zorrilla, et al. (1994). "Neuropsychological functioning in siblings discordant for schizoophrenia and healthy-volunteers." Archives of General Psychiatry **51**(8): 651-661.
Canon, M., P. B. Jones, et al. (2002). "Obstetric complications and schizophrenia: historical and meta-analytic review." American Journal of Psychiatry **159**: 1080-1092.
Cantor-Graae, E., B. Ismail, et al. (2000). "Are neurological abnormalities in schizophrenic patients and their siblings the result of perinatal trauma?" Acta Psychiatrica Scandinavica **101**(2): 142-147.
Cantor-Graae, E., T. F. McNeil, et al. (1994). "Are Neurological Abnormalities in Well Discordant Monozygotic Co-Twins of Schizophrenic Subjects the Result of Perinatal Trauma." American Journal of Psychiatry **151**(8): 1194-1199.

Cantor-Graae, E., T. F. McNeil, et al. (1994). "Are neurological abnormalities in well discordant monozygotic co-twins of schizophrneia subjects the result of perinatal tramua." American Journal of Psychiatry **151**(8): 1194-1199.
Cantor-Graae, E., T. F. McNeil, et al. (1994). "Link between pregnancy complications and minor physical anomalies in monozygotic twins discordant for schizophrenia." American Journal of Psychiatry **151**(8): 1188-1193.
Cantorgraae, E., T. F. McNeil, et al. (1994). "ARE NEUROLOGICAL ABNORMALITIES IN WELL DISCORDANT MONOZYGOTIC CO-TWINS OF SCHIZOPHRENIC SUBJECTS THE RESULT OF PERINATAL TRAUMA." American Journal of Psychiatry **151**(8): 1194-1199.
Cardno, A. G., E. J. Marshall, et al. (1999). "Heritability estimates for psychotic disorders - The Maudsley Twin psychosis series." Archives of General Psychiatry **56**(2): 162-168.
Cardno, A. G., E. J. Marshall, et al. (1999). "Heritability estimates for psychotic disorders." Archives of General Psychiatry **56**: 162-168.
Cardno, A. G., F. V. Rijsdijk, et al. (2002). "A twin study of genetic relationships between psychotic symptoms." American Journal of Psychiatry **159**(4): 539-545.
Cardno, A. G., P. C. Sham, et al. (2002). "Heritability of Schneider's first-rank symptoms." British Journal of Psychiatry **180**: 35-38.
Cardno, A. G., P. C. Sham, et al. (2001). "Twin study of symptom dimensions in psychoses." British Journal of Psychiatry **179**: 39-45.
Carmelli, D., C. DeCarli, et al. (1998). "Evidence for genetic variance in white matter hyperintensity volume in normal elderly male twins." Stroke **29**(6): 1177-1181.
Casanova, M. F., R. D. Sanders, et al. (1990). "MORPHOMETRY OF THE CORPUS-CALLOSUM IN MONOZYGOTIC TWINS DISCORDANT FOR SCHIZOPHRENIA - A MAGNETIC-RESONANCE-IMAGING STUDY." Journal of Neurology Neurosurgery and Psychiatry **53**(5): 416-421.
Caspi, A., T. E. Moffitt, et al. (2005). "Moderation of the effect of adolescent-onset cannabis use on adult psychosis by a functional polymorphism in the catechol-O-methyltransferase gene: Longitudinal evidence of a gene X environment interaction." Biological Psychiatry **57**(10): 1117-1127.
Chakos, M. H., J. A. Lieberman, et al. (1994). "INCREASE IN CAUDATE NUCLEI VOLUMES OF FIRST-EPISODE SCHIZOPHRENIC-PATIENTS TAKING ANTIPSYCHOTIC-DRUGS." American Journal of Psychiatry **151**(10): 1430-1436.
Chan, R. C. K., T. Xu, et al. (2010). "Neurological Soft Signs in Schizophrenia: A Meta-analysis." Schizophrenia Bulletin **36**(6): 1089-1104.
Chauhan, S. P., J. A. Scardo, et al. (2010). "Twins: prevalence, problems, and preterm births." American Journal of Obstetrics and Gynecology **203**(4): 305-315.
Cheek, F. E. (1964). "THE SCHIZOPHRENOGENIC MOTHER IN WORD AND DEED." Family Process **3**(1): 155-177.
Chen, Y. L. R., Y. H. E. Chen, et al. (2000). "Semantic verbal fluency deficit as a familial trait marker in schizophrenia." Psychiatry Research **95**: 133-148.
Collip, D., I. Myin-Germeys, et al. (2008). "Does the concept of "sensitization" provide a plausible mechanism for the putative link between the environment and schizophrenia?" Schizophrenia Bulletin **34**(2): 220-225.
Costafreda, S. G., C. H. Y. Fu, et al. (2009). "Increased Inferior Frontal Activation During Word Generation: A Marker of Genetic Risk for Schizophrenia but not Bipolar Disorder?" Human Brain Mapping **30**(10): 3287-3298.

Cougnard, A., M. Marcelis, et al. (2007). "Does normal developmental expression of psychosis combine with environmental risk to cause persistence of psychosis? A psychosis proneness-persistence model." Psychological Medicine **37**(4): 513-527.
Craddock, N., M. C. O'Donovan, et al. (2007). "Phenotypic and genetic complexity of psychosis - Invited commentary on ... Schizophrenia: a common disease caused by multiple rare alleles." British Journal of Psychiatry **190**: 200-203.
Crawford, J. R., M. C. Obonsawin, et al. (1993). "Frontal lobe impairment in schizophrenia -relationships to intellectual-functioning." Psychological Medicine **23**(3): 787-790.
Crow, T. J. (1999). "Twin studies of psychosis and the genetics of cerebral asymmetry." British Journal of Psychiatry **175**: 399-401.
Crow, T. J. (2000). "Schizophrenia as the price that Homo sapiens pays for language: a resolution of the central paradox in the origin of the species." Brain Research Reviews **31**(2-3): 118-129.
Curtis, V. A., E. Bullmore, et al. (1998). "Attenuated frontal activation during a verbal fluency task in patients wiht schizophrenia." American Journal of Psychiatry **155**: 1056-1063.
Curtis, V. A., E. T. Bullmore, et al. (1999). "Attenuated frontal activation in schizophrenia may be task dependent." Schizophrenia Research **37**(1): 35-44.
Damasio, H., T. J. Grabowski, et al. (1996). "A neural basis for lexical retrieval." Nature **380**(6574): 499-505.
Davatzikos, C., D. G. Shen, et al. (2005). "Whole-brain morphometric study of schizophrenia revealing a spatially complex set of focal abnormalities." Archives of General Psychiatry **62**(11): 1218-1227.
Davidson, M., A. Reichenberg, et al. (1999). "Behavioral and intellectual markers for schizophrenia in apparently healthy male adolescents." American Journal of Psychiatry **156**(9): 1328-1335.
Davis, J. O. and H. S. Bracha (1996). "Prenatal growth markers in schizophrenia: A monozygotic co-twin control study." American Journal of Psychiatry **153**(9): 1166-1172.
Davis, J. O. and J. A. Phelps (1995). "TWINS WITH SCHIZOPHRENIA - GENES OR GERMS." Schizophrenia Bulletin **21**(1): 13-18.
Davis, J. O., J. A. Phelps, et al. (1995). "PRENATAL DEVELOPMENT OF MONOZYGOTIC TWINS AND CONCORDANCE FOR SCHIZOPHRENIA." Schizophrenia Bulletin **21**(3): 357-366.
Dazzan, P., K. D. Morgan, et al. (2005). "Different effects of typical and atypical antipsychotics on grey matter in first episode psychosis: the AE SOP study." Neuropsychopharmacology **30**(4): 765-774.
Dazzan, P., K. D. Morgan, et al. (2004). "The structural brain correlates of neurological soft signs in AE SOP first-episode psychoses study." Brain **127**: 143-153.
Deb-Rinker, P., T. A. Klempan, et al. (1999). "Molecular characterization of a MSRV-like sequence identified by RDA from monozygotic twin pairs discordant for schizophrenia." Genomics **61**(2): 133-144.
Denbow, M. L. and N. M. Fisk (1996). "Chorionicity and twins." Current Obstetrics and Gynaecology **6**: 212-219.
Dickey, C. C., R. W. McCarley, et al. (2001). "MRI measures of prefrontal gray and white matter in males and females diagnosed with schizotypal personality disorder." Schizophrenia Research **49**(1-2): 153-153.

Dickey, C. C., R. W. McCarley, et al. (2007). "MRI abnormalities of the hippocampus and cavum septi pellucidi in females with schizotypal personality disorder." Schizophrenia Research **89**(1-3): 49-58.
Dilalla, D. L. and Gottesman, II (1995). "Normal personality-characteristics in identical-twins discordant for schizophrenia." Journal of Abnormal Psychology **104**(3): 490-499.
Docherty, N. M. and Gottesman, II (2000). "A twin study of communication disturbances in schizophrenia." Journal of Nervous and Mental Disease **188**(7): 395-401.
Done, D. J., T. J. Crow, et al. (1994). "Childhood Antecedents of Schizophrenia and Affective-Illness - Social-Adjustment at Ages 7 and 11." British Medical Journal **309**(6956): 699-703.
Dworkin, R. H., J. A. Lewis, et al. (1994). "Social competence deficits in adolescents at risk for schizophrenia." Journal of Nervous and Mental Disease **182**(2): 103-108.
Eaves, L., D. Foley, et al. (2003). "Has the "equal environments" assumption been tested in twin studies?" Twin Research **6**(6): 486-489.
Ebmeier, K. P., D. H. R. Blackwood, et al. (1993). "SINGLE-PHOTON EMISSION COMPUTED-TOMOGRAPHY WITH TC-99M-EXAMETAZIME IN UNMEDICATED SCHIZOPHRENIC-PATIENTS." Biological Psychiatry **33**(7): 487-495.
Eckert, M. A., C. M. Leonard, et al. (2002). "The epigenesis of planum temporale asymmetry in twins." Cerebral Cortex **12**(7): 749-755.
Egan, M. F., T. M. Hyde, et al. (2001). "Relative risk of neurological signs in siblings of patients with schizophrenia." American Journal of Psychiatry **158**(11): 1827-1834.
Elvevag, B., D. M. Weinstock, et al. (2001). "A comparison of verbal fluency tasks in schizophrenic patients and normal controls." Schizophrenia Research **51**(2-3): 119-126.
Endicott, J. and R. L. Spitzer (1978). "A diagnostic interview: the schedule for affective disorders and schizophrenia." Arch Gen Psychiatry **35**(7): 837-844.
Ettinger, A. Schmechtig, et al. (2010). "Prefrontal and Striatal Volumes in Monozygotic Twins Concordant and Discordant for Schizophrenia " Schizophrenia Bulletin **first published online June 10, 2010 doi:10.1093/schbul/sbq060**.
Ettinger, U., M. Picchioni, et al. (2006). "Antisaccade performance in monozygotic twins discordant for schizophrenia: The Maudsley twin study." American Journal of Psychiatry **163**(3): 543-545.
Ettinger, U., M. Picchioni, et al. (2007). "Magnetic resonance imaging of the thalamus and adhesio interthalamica in twins with schizophrenia." Archives of General Psychiatry **64**(4): 401-409.
Falret, J. (1878). "La folie circulaire ou folie à formes alternes." Archives Génerales de Médecine.
Farmer, A. E., P. McGuffin, et al. (1984). "Searching for the split in schizophrenia - a twin study perspective." Psychiatry Research **13**(2): 109-118.
Farmer, A. E., P. McGuffin, et al. (1987). "TWIN CONCORDANCE FOR DSM-III SCHIZOPHRENIA - SCRUTINIZING THE VALIDITY OF THE DEFINITION." Archives of General Psychiatry **44**(7): 634-641.
Fearon, P., J. B. Kirkbride, et al. (2006). "Incidence of schizophrenia and other psychoses in ethnic minority groups: results from the MRC AESOP study." Psychological Medicine **36**(11): 1541-1550.

Featherstone, R. E., S. Kapur, et al. (2007). "The amphetamine-induced sensitized state as a model of schizophrenia." Progress in Neuro-Psychopharmacology & Biological Psychiatry **31**(8): 1556-1571.
Fischer, M. (1971). "Psychoses in the offspring of schizophrenic monozygotic twins and their normal co-twins." British Journal of Psychiatry **118**: 43-52.
Fischer, M. (1973). "Genetic and environmental factors in schizophrenia A study of schizophrenic twins and their families." Acta Psychiatrica Scandinavica Supplementum **238**: 5-152.
Fischer, M. (1973). "Genetic and environmental factors in schizophrenia. A study of schizophrenic twins and their families." Acta Psychiatr Scand Suppl **238**: 9-142.
Fischer, M. and Christen.A (1973). "Genetic and Environmental Factors in Schizophrenia - Study of Schizophrenic Twins and Their Families." Acta Psychiatrica Scandinavica: 1-158.
Fletcher, P. C., P. J. McKenna, et al. (1998). "Brain activations in schizophrenia during a graded memory task studied with functional neuroimaging." Archives of General Psychiatry **55**(11): 1001-1008.
Flint, J. I. and M. R. Munafo (2007). "The endophenotype concept in psychiatric genetics." Psychological Medicine **37**: 163-180.
Flyckt, L., O. Sydow, et al. (1999). "Neurological signs and psychomotor performance in patients with schizophrenia, their relatives and healthy controls." Psychiatry Research **86**(2): 113-129.
Foerster, A., S. Lewis, et al. (1991). "Premorbid Adjustment and Personality in Psychosis - Effects of Sex and Diagnosis." British Journal of Psychiatry **158**: 171-176.
Franzek, E. and H. Beckmann (1998). "Different genetic background of schizophrenia spectrum psychoses: A twin study." American Journal of Psychiatry **155**(1): 76-83.
Friedhoff, A. J., J. C. Miller, et al. (1995). "A SUBTRACTED PROBE DERIVED FROM LYMPHOCYTES OF TWINS DISCORDANT FOR SCHIZOPHRENIA HYBRIDIZES TO SELECTIVE AREAS OF RAT-BRAIN." Biological Psychiatry **37**(2): 127-131.
Friman, O., M. Borga, et al. (2003). "Adaptive analysis of fMRI data." Neuroimage **19**(3): 837-845.
Frith, C. D., K. J. Friston, et al. (1995). "Regional Brain Activity in Chronic-Schizophrenic Patients During the Performance of a Verbal Fluency Task." British Journal of Psychiatry **167**: 343-349.
Frommreichmann, F. (1954). "PSYCHOTHERAPY OF SCHIZOPHRENIA." American Journal of Psychiatry **111**(6): 410-419.
Fu, C. H. Y., K. Morgan, et al. (2001). "A functional magnetic resonance imaging study of overt letter verbal fluency using a clustered acquisition sequence: Greater anterior cingulate activation with increases task demand." Neuroimage **17**: 871-879.
Fu, C. H. Y., J. Suckling, et al. (2005). "Effects of psychotic state and task demand on prefrontal function in schizophrenia: An fMRI study of overt verbal fluency." American Journal of Psychiatry **162**(3): 485-494.
Fuller Torry, E., A. E. Bowler, et al. (1994). Schizophrenia and Manic-Depressive Disorder, The Biological Roots of Mental Illness as Revealed by the Landmark Study of Identical Twins. New York, Basic Books.
Gatz, M., N. L. Pedersen, et al. (2000). "Defining discordance in twin studies of risk and protective factors for late life disorders." Twin Res **3**(3): 159-164.

Geschwind, D. H., B. L. Miller, et al. (2002). "Heritability of lobar brain volumes in twins supports genetic models of cerebral laterality and handedness." Proceedings of the National Academy of Sciences of the United States of America **99**(5): 3176-3181.
Gilmore, J. H., D. O. Perkins, et al. (1996). "Fetal brain development of twins assessed in utero by ultrasound: Implications for schizophrenia." Schizophrenia Research **19**(2-3): 141-149.
Gilvarry, C. M., A. Russell, et al. (2001). "Neuropsychological performance and spectrum personality traits in the relatives of patients with schizophrenia and affective psychosis." Psychiatry Research **101**(2): 89-100.
Gilvarry, C. M., A. Russell, et al. (2001). "Verbal fluency in patients with schizophrenia and affective psychoses and their first-degree realtives." Psychological Medicine **31**: 695-704.
Gilvarry, C. M., A. Russell, et al. (2001). "Verbal fluency in patients with schizophrenia and affective psychoses and their first-degree relatives." Psychological Medicine **31**(4): 695-704.
Gleissner, U. and C. E. Elger (2001). "The hippocampal contribution to verbal fluency in patients with temporal lobe epilepsy." Cortex **37**(1): 55-63.
Goldberg, T. E., J. D. Ragland, et al. (1990). "NEUROPSYCHOLOGICAL ASSESSMENT OF MONOZYGOTIC TWINS DISCORDANT FOR SCHIZOPHRENIA." Archives of General Psychiatry **47**(11): 1066-1072.
Goldberg, T. E., E. F. Torrey, et al. (1994). "RELATIONS BETWEEN NEUROPSYCHOLOGICAL PERFORMANCE AND BRAIN MORPHOLOGICAL AND PHYSIOLOGICAL MEASURES IN MONOZYGOTIC TWINS DISCORDANT FOR SCHIZOPHRENIA." Psychiatry Research-Neuroimaging **55**(1): 51-61.
Goldberg, T. E., E. F. Torrey, et al. (1995). "GENETIC RISK OF NEUROPSYCHOLOGICAL IMPAIRMENT IN SCHIZOPHRENIA - A STUDY OF MONOZYGOTIC TWINS DISCORDANT AND CONCORDANT FOR THE DISORDER." Schizophrenia Research **17**(1): 77-84.
Goldberg, T. E., E. F. Torrey, et al. (1993). "LEARNING AND MEMORY IN MONOZYGOTIC TWINS DISCORDANT FOR SCHIZOPHRENIA." Psychological Medicine **23**(1): 71-85.
Goldman, A. L., L. Pezawas, et al. (2009). "Widespread Reductions of Cortical Thickness in Schizophrenia and Spectrum Disorders and Evidence of Heritability." Archives of General Psychiatry **66**(5): 467-+.
Goldman, A. L., L. Pezawas, et al. (2008). "Heritability of brain morphology related to schizophrenia: A large-scale automated magnetic resonance imaging segmentation study." Biological Psychiatry **63**: 475-483.
Goldman, A. L., L. Pezawas, et al. (2008). "Heritability of brain morphology related to schizophrenia: A large-scale automated magnetic resonance imaging segmentation study." Biological Psychiatry **63**(5): 475-483.
Goldstein, K. E., E. A. Hazlett, et al. (2009). "Smaller superior temporal gyrus volume specificity in schizotypal personality disorder." Schizophrenia Research **112**(1-3): 14-23.
Good, C. D., I. S. Johnsrude, et al. (2001). "A voxel-based morphometric study of ageing in 465 normal adult human brains." NeuroImage **14**(1): 21-36.
Gottesman, II and A. Bertelsen (1989). "Confirming unexpressed genotypes for schizophrenia - Risks in the offspring of Fischer's identical and fraternal discordant trwins." Archives of General Psychiatry **46**(10): 867-872.

Gottesman, II and T. D. Gould (2003). "The endophenotype concept in psychiatry: Etymology and strategic intentions." American Journal of Psychiatry **160**(4): 636-645.
Goulding, S. M., A. S. Leiner, et al. (2008). "Family strengths: a potential determinant of the duration of untreated psychosis among hospitalized African-American first-episode patients." Early Intervention in Psychiatry **2**(3): 162-168.
Gourovitch, M. L., T. E. Goldberg, et al. (1996). "Verbal fluency deficits in patients with schizophrenia: Semantic fluency is differentially impaired as compared with phonologic fluency." Neuropsychology **10**(4): 573-577.
Griffiths, T. D., T. Sigmundsson, et al. (1998). "Neurological abnormalities in familial and sporadic schizophrenia." Brain **121**: 191-203.
Gringras, P. and W. Chen (2001). "Mechanisms for differences in monozygous twins." Early Human Development **64**(2): 105-117.
Grove, W. M., B. S. Lebow, et al. (1991). "Familial prevalence and coaggregation of schizotypy indicators: a multitrait family study
" Journal of Abnormal Psychology **100**(2): 115-121.
Gupta, S., N. C. Andreasen, et al. (1995). "Neurological Soft Signs in Neuroleptic-Naive and Neuroleptic- Treated Schizophrenic-Patients and in Normal Comparison Subjects." American Journal of Psychiatry **152**(2): 191-196.
Gupta, S., R. Rajaprabhakaran, et al. (1995). "Premorbid Adjustment as a Predictor of Phenomenological and Neurobiological Indexes in Schizophrenia." Schizophrenia Research **16**(3): 189-197.
Gur, R. C. and R. E. Gur (1995). "Hypofrontality in schizophrenia: RIP." Lancet **345**: 1383-1384.
Gur, R. E., B. I. Turetsky, et al. (2000). "Temporolimbic volume reductions in schizophrenia." Archives of General Psychiatry **57**(8): 769-775.
Gurd, J. M., K. Amunts, et al. (2002). "Posterior parietal cortex is implicated in continuous switching between verbal fluency tasks: an fMRI study with clinical implications." Brain **125**: 1024-1038.
Hall, J. G. (1996). "Twinning: Mechanisms and genetic implications." Current Opinion in Genetics & Development **6**(3): 343-347.
Hall, M. H., F. Rijsdijk, et al. (2007). "Substantial shared genetic influences on schizophrenia and event-related potentials." American Journal of Psychiatry **164**(5): 804-812.
Hans, S. L., J. G. Auerbach, et al. (2000). "Social adjustment of adolescents at risk for schizophrenia: The Jerusalem Infant Development Study." Journal of the American Academy of Child and Adolescent Psychiatry **39**(11): 1406-1414.
Hans, S. L., J. G. Auerbach, et al. (2005). "Development from birth to adolescence of children at-risk for schizophrenia." Journal of Child and Adolescent Psychopharmacology **15**(3): 384-394.
Harrison, P. J. and S. L. Eastwood (2001). "Neuropathological studies of synaptic connectivity in the hippocampal formation in schizophrenia." Hippocampus **11**(5): 508-519.
Hay, D. A., N. G. Martin, et al. (2001). "Phenotypic and genetic analysis of a short measure of psychosis-proness in a large-scale Australian twin study." Twin Research **4**: 30-40.
Hecker, E. (1871). "Die Henrephrenie." Archiv fur pathologische Anatomie und Physiologie und fur kliniche Medizin **25**: 394-429.
Heckers, S., S. L. Rauch, et al. (1998). "Impaired recruitment of the hippocampus during conscious recollection in schizophrenia." Nature Neuroscience **1**(4): 318-323.

Henquet, C., R. Murray, et al. (2005). "The environment and schizophrenia: The role of cannabis use." Schizophrenia Bulletin **31**(3): 608-612.
Hettema, J. M., M. C. Neale, et al. (1995). "Physical similarity and the equal-environments assumption in twin studies of psychiatric-disorders." Behavior Genetics **25**(4): 327-335.
Hirvonen, J., T. G. M. van Erp, et al. (2005). "Increased caudate dopamine D-2 receptor availability as a genetic marker for schizophrenia." Archives of General Psychiatry **62**(4): 371-378.
Hoffer, A. and W. Pollin (1970). "SCHIZOPHRENIA IN NAS-NRC PANEL OF 15,909 VETERAN TWIN PAIRS." Archives of General Psychiatry **23**(5): 469-&.
Holzman, P. S., E. Kringlen, et al. (1980). "DEVIANT EYE TRACKING IN TWINS DISCORDANT FOR PSYCHOSIS - A REPLICATION." Archives of General Psychiatry **37**(6): 627-631.
Holzman, P. S., E. Kringlen, et al. (1978). "SMOOTH PURSUIT EYE-MOVEMENTS IN TWINS DISCORDANT FOR SCHIZOPHRENIA." Journal of Psychiatric Research **14**(1-4): 111-120.
Holzman, P. S., E. Kringlen, et al. (1977). "ABNORMAL-PURSUIT EYE-MOVEMENTS IN SCHIZOPHRENIA." Archives of General Psychiatry **34**(7): 802-805.
Holzman, P. S., D. L. Levy, et al. (1997). "Smooth pursuit eye tracking in twins - A critical commentary." Archives of General Psychiatry **54**(5): 429-431.
Honea, R., T. J. Crow, et al. (2005). "Regional deficits in brain volume in schizophrenia: A meta-analysis of voxel-based morphometry studies." American Journal of Psychiatry **162**(12): 2233-2245.
Honea, R. A., A. Meyer-Lindenberg, et al. (2008). "Is gray matter volume an intermediate phenotype for schizophrenia? A voxel-based morphometry study of patients with schizophrenia and their healthy siblings." Biological Psychiatry **63**: 465-474.
Honea, R. A., A. Meyer-Lindenberg, et al. (2008). "Is gray matter volume an intermediate phenotype for schizophrenia? A voxel-based morphometry study of patients with schizophrenia and their healthy siblings." Biological Psychiatry **63**(5): 465-474.
Hoptman, M. J., J. Nierenberg, et al. (2008). "A DTI study of white matter microstructure in individuals at high genetic risk for schizophrenia." Schizophrenia Research **106**(2-3): 115-124.
Horwitz, A. V., T. M. Videon, et al. (2003). "Rethinking twins and environments: Possible social sources for assumed genetic influences in twin research." Journal of Health and Social Behavior **44**(2): 111-129.
Howes, O. D., C. McDonald, et al. (2004). "Pathways to schizophrenia: the impact of environmental factors." International Journal of Neuropsychopharmacology **7**((Supplement 1)): S7-S13.
Hui, C. L. M., G. H. Y. Wong, et al. (2009). "Potential Endophenotype for Schizophrenia: Neurological Soft Signs." Annals Academy of Medicine Singapore **38**(5): 408-413.
Hulshoff Pol, H. E., R. G. H. Brans, et al. (2004). "Gray and white matter volume abnormalities in monozygotic and same-gender dizygotic twins discordant for schizophrenia." Biological Psychiatry **55**(2): 126-130.
Hulshoff Pol, H. E., H. G. Schnack, et al. (2002). "Volume changes in gray matter in patients with schizophrenia." American Journal of Psychiatry **159**(2): 244-250.

Hulshoff Pol, H. E., H. G. Schnack, et al. (2006). "Gray and white matter density changes in monozygotic and same-sex dizygotic twins discordant for schizophrenia using voxel-based morphometry." Neuroimage **31**(2): 482-488.
Hulshoff Pol, H. E., H. G. Schnack, et al. (2001). "Focal gray matter density changes in schizophrenia." Archives of General Psychiatry **58**(12): 1118-1125.
Imaizumi, Y. (2003). "A comparative study of zygotic twinning and triplet rates in eight countries, 1972-1999." Journal of Biosocial Science **35**(2): 287-302.
Ismail, B., E. Cantor-Graae, et al. (1998). "Neurological abnormalities in schizophrenic patients and their siblings." American Journal of Psychiatry **155**(1): 84-89.
Jablensky, A. (2000). "Epidemiology of schizophrenia: the global burden of disease and disability." European Archives of Psychiatry and Clinical Neuroscience **250**(6): 274-285.
Jackson, D. D., Ed. (1960). The Etiology of Schizophrenia. New York, Basic Books, Inc.
Jakobsen, K. D., T. Hansen, et al. (2007). "Diagnostic stability among chronic patients with functional psychoses: an epidemiological and clinical study." BMC Psychiatry **7**.
Jang, K. L., T. S. Woodward, et al. (2005). "the genetic and environmental basis of the relationship between schizotypy and personality." The Journal of Nervous and Mental Disease **193**(3): 153-159.
Job, D. E., H. C. Whalley, et al. (2005). "Grey matter changes over time in high risk subjects developing schizophrenia." NeuroImage **25**(4): 1023-1030.
Johnson, J. K., M. O. Huttunen, et al. (2003). "The relationship between schizotypal symptoms, genetic risk for schizophrenia, and neurocognitive deficits: A twin study." Schizophrenia Research **60**(1): 82-82.
Johnson, J. K., A. Tuulio-Henriksson, et al. (2003). "Do schizotypal symptoms mediate the relationship between genetic risk for schizophrenia and impaired neuropsychological performance in co-twins of schizophrenic patients?" Biological Psychiatry **54**(11): 1200-1204.
Johnstone, E. C., K. P. Ebmeier, et al. (2005). "Predicting schizophrenia: findings from the Edinburgh high-risk study." British Journal of Psychiatry **186**: 18-25.
Jones, H. E. (1955). "Perceived differences among twins." Eugenics Quarterly **2**: 98-102.
Joseph, J. (1998). "The equal environment assumption of the classical twin method: A critical analysis." Journal of Mind and Behavior **19**(3): 325-358.
Joyce, E. M., S. L. Collinson, et al. (1996). "Verbal fluency in schizophrenia: Relationship with executive function, semantic memory and clinical alogia." Psychological Medicine **26**(1): 39-49.
Kahlbaum, K. L. (1863). Die Gruppierung der psychischen Krankhieten und die Einterlung der Seelenstoerungen. Danzig, AW Kafemann.
Kanaan, R. A. A., J. S. Kim, et al. (2005). "Diffusion tensor imaging in schizophrenia." Biological Psychiatry **58**(12): 921-929.
Kaufman, M. H. (2004). "The embryology of conjoined twins." Childs Nervous System **20**: 508-525.
Kay, S. R., L. A. Opler, et al. (1989). "THE POSITIVE AND NEGATIVE SYNDROME SCALE (PANSS) - RATIONALE AND STANDARDIZATION." British Journal of Psychiatry, Vol 155, Supp No. 7: 59-65.
Keller, M. C., W. L. Coventry, et al. (2005). "Widespread evidence for non-additive genetic variation in Cloninger's and Eysenck's personality dimensions using a twin plus sibling design." Behavior Genetics **35**(6): 707-721.
Kelly, B. D., D. Cotter, et al. (2004). "Neurological soft signs and dermatoglyphic anomalies in twins with schizophrenia." European Psychiatry **19**(3): 159-163.

Kendler, K. S. and S. R. Diehl (1993). "The genetics of schizophrrenia - a current, genetic-epidemiologic perspective." Schizophrenia Bulletin **19**(2): 261-285.
Kendler, K. S. and J. Hewitt (1992). "The Structure of Self-Report Schizotypy in Twins." Journal of Personality Disorders **6**(1): 1-17.
Kendler, K. S., M. McGuire, et al. (1993). "The Roscommon Family Study I. Methods, diagnosis of probands, and risk of schizophrenia in relatives." Arch Gen Psychiatry **50**: 527-540.
Kendler, K. S., M. McGuire, et al. (1993). "The Roscommon Family Study .1. Methods, Diagnosis of Probands, and Risk of Schizophrenia in Relatives." Archives of General Psychiatry **50**(7): 527-540.
Kendler, K. S., M. McGuire, et al. (1995). "Schizotypal symptoms and signs in the Roscommon Family Study. Their factor structure and familial relationship with psychotic and affective disorders." Arch Gen Psychiatry **52**: 296-303.
Kendler, K. S. and M. C. Neale (2010). "Endophenotype: a conceptual analysis." Molecular Psychiatry **15**(8): 789-797.
Kendler, K. S., M. C. Neale, et al. (1993). "A test of the equal-environments assumption in twin studies of psychiatric-illness." Behavior Genetics **23**(1): 21-27.
Kendler, K. S., M. C. Neale, et al. (1993). "A test of the equal environments assumption in twin studies of psychiatric-illness." Behavior Genetics **23**(1): 21-27.
Kendler, K. S., M. C. Neale, et al. (1994). "Parental treatment and the equal environment assumption in twin studies of psychiatric-illness." Psychological Medicine **24**(3): 579-590.
Kendler, K. S. and C. D. Robinette (1983). "Schizophrenia in the National Academy of Sciences- National Research Council Twins Registry- A 16 year update." American Journal of Psychiatry **140**(12): 1551-1563.
Keshavan, M. S., E. Dick, et al. (2002). "Decreased left amygdala and hippocampal volumes in young offspring at risk for schizophrenia." Schizophrenia Research **58**(2-3): 173-183.
Keshavan, M. S., D. M. Montrose, et al. (1997). "Magnetic resonance imaging and spectroscopy in offspring at risk for schizophrenia: Preliminary studies." Progress in Neuro-Psychopharmacology & Biological Psychiatry **21**(8): 1285-1295.
Keshavan, M. S., R. D. Sanders, et al. (2003). "Diagnostic specificity and neuroanatomical validity of neurological abnormalities in first-episode psychoses." American Journal of Psychiatry **160**(7): 1298-1304.
Kinney, D. K., D. A. Yurgelun Todd, et al. (1991). "Hard Neurologic Signs and Psychopathology in Relatives of Schizophrenic-Patients." Psychiatry Research **39**(1): 45-53.
Klaning, U. (1999). "Greater occurrence of schizophrenia in dizygotic but not monozygotic twins - Register-based study." British Journal of Psychiatry **175**: 407-409.
Klaning, U., P. B. Mortensen, et al. (1996). "Increased occurrence of schizophrenia and other psychiatric illnesses among twins." British Journal of Psychiatry **168**(6): 688-692.
Konrad, A. and G. Winterer (2008). "Disturbed structural connectivity in schizophrenia - Primary factor in pathology or epiphenomenon?" Schizophrenia Bulletin **34**(1): 72-92.
Koo, M. S., C. C. Dickey, et al. (2006). "Smaller neocortical gray matter and larger sulcal cerebrospinal fluid volumes in neuroleptic-naive women with schizotypal personality disorder." Archives of General Psychiatry **63**(10): 1090-1100.

Koolschijn, P., N. E. M. van Haren, et al. (2008). "Hypothalamus volume in twin pairs discordant for schizophrenia." European Neuropsychopharmacology **18**(4): 312-315.
Kopala, L. C., K. P. Good, et al. (1998). "Olfactory function in monozygotic twins discordant for schizophrenia." American Journal of Psychiatry **155**(1): 134-136.
Koutsouleris, N., E. M. Meisenzahl, et al. (2009). "Use of Neuroanatomical Pattern Classification to Identify Subjects in At-Risk Mental States of Psychosis and Predict Disease Transition." Archives of General Psychiatry **66**(7): 700-712.
Kraepelin, E. (1899). Psychiatrie. Ein Lehrbuch fur Studirende und Aertze. Leipzig, Barth.
Kraepelin, E. (1913). Dementia Praecox. Edinburgh, E and S Livingstone.
Kraepelin, E. (1919). Dementia Praecox. Edinburgh, Livingstone.
Kremen, W. S., S. V. Faraone, et al. (1998). "Sex differences in self-reported schizotypal traits in relatives of schizophrenic probands." Schizophrenia Research **34**(1-2): 27-37.
Kringlen, E. (1967). Heredity and environment in the functional psychoses. Oslo, Universitetsforlaget.
Kringlen, E. (1967). Heredity and the environment in the functional psychoses. London, William Heinmann.
Kringlen, E. and G. Cramer (1989). "Offspring of monozygotic twins discordant for schizophrenia." Archives of General Psychiatry **46**(10): 873-877.
Kruyer, H., M. Mila, et al. (1994). "FRAGILE X SYNDROME AND THE (CGG)(N) MUTATION - 2 FAMILIES WITH DISCORDANT MZ TWINS." American Journal of Human Genetics **54**(3): 437-442.
Kubicki, M., R. McCarley, et al. (2007). "A review of diffusion tensor imaging studies in schizophrenia." Journal of Psychiatric Research **41**(1-2): 15-30.
Kubicki, M., M. E. Shenton, et al. (2002). "Voxel-based morphornetric analysis of gray matter in first episode schizophrenia." NeuroImage **17**(4): 1711-1719.
Kulkarni, M. L., C. Sureshkumar, et al. (1994). "Conjoined twins." Indian Pediatr **31**(8): 1017-1024.
Kunugi, H., M. Tsukue, et al. (1999). "A case of a monozygotic twin pair discordant for schizophrenia a possible link between prenatal hypoxia and neurodevelopmental impairment." Schizophrenia Research **36**(1-3): 91-91.
Kunugi, H., T. Urushibara, et al. (2003). "Prenatal underdevelopment and schizophrenia: A case report of monozygotic twins." Psychiatry and Clinical Neurosciences **57**(3): 271-274.
Laing, R. D. (1964). "What is schizophrenia." New Left Review(28): 63-68.
Laing, R. D. (1983). The Politics of Experience. Harmondsworth, Penguin.
Laruelle, M. (1998). The role of endogenous sensitization in the pathophysiology of schizophrenia: implications from recent brain imaging studies. Nobel Symposium 111: Schizophrenia - Pathophysiological Mechanisms, Stockholm, Sweden, Elsevier Science Bv.
Laruelle, M. (2000). "The role of endogenous sensitization in the pathophysiology of schizophrenia: implications from recent brain imaging studies." Brain Research Reviews **31**(2-3): 371-384.
Laurent, A., M. Biloa-Tang, et al. (2000). "Executive/attentional performance and measures of schizotypy in patients with schizophrenia and in their nonpsychotic first-degree relatives." Schizophrenia Research **46**(2-3): 269-283.

Lavrentieva, I., N. E. Broude, et al. (1999). "High polymorphism level of genomic sequences flanking insertion sites of human endogenous retroviral long terminal repeats." Febs Letters **443**(3): 341-347.
Lawrie, S. M. and S. S. Abukmeil (1998). "Brain abnormality in schizophrenia - A systematic and quantitative review of volumetric magnetic resonance imaging studies." British Journal of Psychiatry **172**: 110-120.
Lawrie, S. M., A. M. McIntosh, et al. (2008). "Brain structure and function changes during the development of schizophrenia: The evidence from studies of subjects at increased genetic risk." Schizophrenia Bulletin **34**(2): 330-340.
Lawrie, S. M., H. C. Whalley, et al. (2001). "Brain structure, genetic liability, and psychotic symptoms in subjects at high risk of developing schizophrenia." Biological Psychiatry **49**(10): 811-823.
Leask, S. J., D. J. Done, et al. (2002). "Adult psychosis, common childhood infections and neurological soft signs in a national birth cohort." British Journal of Psychiatry **181**: 387-392.
Lehmann, E. L. (1975). Nonparametrics: Statistical methods based on ranks. San Francisco, Holden-Day, Inc.
Lewine, R. R., N. F. Watt, et al. (1980). "Childhood social competence in functionally disordered psychiatric patients and in normals." Journal of Abnormal Psychology **89**(2): 132-138.
Lewis, S. (1996). "Schizophrenia in twins." British Journal of Psychiatry **169**(4): 522-523.
Lewis, S. W., B. Chitkara, et al. (1989). "HAND PREFERENCE IN PSYCHOTIC TWINS." Biological Psychiatry **25**(2): 215-221.
Lewis, S. W., B. Chitkara, et al. (1987). "FAMILY HISTORY AND BIRTH-WEIGHT IN MONOZYGOTIC TWINS CONCORDANT AND DISCORDANT FOR PSYCHOSIS." Acta Geneticae Medicae Et Gemellologiae **36**(2): 267-273.
Liddle, P. F. (1987). "The symptoms of chronic schizophrenia: A re-examination of the positive-negative dichotomy." British Journal of Psychiatry **151**: 145-151.
Liddle, P. F. and T. R. E. Barnes (1990). "Syndromes of chronic schizophrenia." British Journal of Psychiatry **157**: 558-561.
Lidz, T., S. Schafer, et al. (1962). "EGO DIFFERENTIATION AND SCHIZOPHRENIC SYMPTOM FORMATION IN IDENTICAL-TWINS." Journal of the American Psychoanalytic Association **10**(1): 74-90.
Lieberman, J. A. (1999). "Is schizophrenia a neurodegenerative disorder? A clinical and neurobiological perspective." Biological Psychiatry **46**(6): 729-739.
Lieberman, J. A. (1999). "Searching for the neuropathology of schizophrenia: Neuroimaging strategies and findings." American Journal of Psychiatry **156**(8): 1133-1136.
Lieberman, J. A., G. D. Tollefson, et al. (2005). "Antipsychotic drug effects on brain morphology in first-episode psychosis." Archives of General Psychiatry **62**(4): 361-370.
Linney, Y. M., R. M. Murray, et al. (2003). "A quantitative genetic analysis of schizotypal personality traits." Psychological Medicine **33**(5): 803-816.
Litman, R. E., E. F. Torrey, et al. (1997). "A quantitative analysis of smooth pursuit eye tracking in monozygotic twins discordant for schizophrenia." Archives of General Psychiatry **54**(5): 417-426.
Lodge, D. J. and A. A. Grace (2007). "Aberrant hippocampal activity underlies the dopamine dysregulation in an animal model of schizophrenia." The Journal of Neuroscience **27**(42): 11424-11430.

Loehlin, J. (1986). "Heredity, Environment, and the Thurstone Temperament Schedule." Behavioral Genetics **16**: 61-73.
Lohmann, G., D. Y. von Cramon, et al. (1999). "Sulcal variability of twins." Cerebral Cortex **9**(7): 754-763.
Luchins, D., W. Pollin, et al. (1980). "LATERALITY IN MONOZYGOTIC SCHIZOPHRENIC TWINS - ALTERNATIVE HYPOTHESIS." Biological Psychiatry **15**(1): 87-93.
Lutkenhoff, E. S., T. G. van Erp, et al. (2010). "Proton MRS in twin pairs discordant for schizophrenia." Molecular Psychiatry **15**(3): 308-318.
Luxenburger, H. (1940). Zwillingsforschung als Methode der Erbforschung beim Menschen. Hanbuch der Erbbiologie der Menschen, Vol II, Methodik Genetik der Gesamptperson. G. Just, K. H. Baner and E. Hanhart. Berlin, Springer**:** 213-248.
Lykken, D. T., M. McGue, et al. (1990). "Does contact lead to similarity or similarlity lead to contact." Behavior Genetics **20**(5): 547-561.
Lyons, M. J., J. L. Bar, et al. (2002). "Nicotine and familial vulnerability to schizophrenia: A discordant twin study." Journal of Abnormal Psychology **111**(4): 687-693.
Lyons, M. J., R. Toomey, et al. (1997). "Psychopathology in twin pairs discordant for schizophrenia." American Journal of Medical Genetics **74**(6): 562-562.
Lytton, H. (1977). "DO PARENTS CREATE, OR RESPOND TO, DIFFERENCES IN TWINS." Developmental Psychology **13**(5): 456-459.
MacDonald, A. W., M. F. Pogue-Geile, et al. (2001). "Genetic and environmental influences on schizotypy: A community based twin study." Schizophrenia Bulletin **27**(1): 47-58.
Machin, G., K. Still, et al. (1996). "Correlations of placental vascular anatomy and clinical outcomes in 69 monochorionic twin pregnancies." American Journal of Medical Genetics **61**(3): 229-236.
Machin, G. A. (1996). "Placental vascular anatomy and twin transfusion syndrome." American Journal of Obstetrics and Gynecology **174**(2): 799-800.
Machin, G. A. (1996). "Some causes of genotypic and phenotypic discordance in monozygotic twin pairs." American Journal of Medical Genetics **61**(3): 216-228.
Mahy, G. E., R. Mallett, et al. (1999). "First-contact incidence rate of schizophrenia on Barbados." British Journal of Psychiatry **175**: 28-33.
Maier, W., D. Lichtermann, et al. (1994). "Personality disorders among the relatives of schizophrenia patients." Schizophrenia Bulletin **20**(3): 481-493.
Malaspina, D., S. Harlap, et al. (2001). "Advancing paternal age and the risk of schizophrenia." Archives of General Psychiatry **58**(4): 361-367.
Malmberg, A., G. Lewis, et al. (1998). "Premorbid adjustment and personality in people with schizophrenia." British Journal of Psychiatry **172**: 308-313.
Marcus, J., S. L. Hans, et al. (1985). "Neurological Dysfunctioning in Offspring of Schizophrenics in Israel and Denmark - a Replication Analysis." Archives of General Psychiatry **42**(8): 753-761.
Mata, I., C. M. Gilvarry, et al. (2003). "Schizotypal personality traits in nonpsychotic relatives are associated with positive symptoms in psychotic probands." Schizophrenia Bulletin **29**(2): 273-283.
Mata, I., M. J. Madoz, et al. (2001). "Olanzapine: concordant response in monozygotic twins with schizophrenia." British Journal of Psychiatry **178**: 86.
Mathalon, D. H., E. V. Sullivan, et al. (2001). "Progressive brain volume changes and the clinical course of schizophrenia in men - A longitudinal magnetic resonance imaging study." Archives of General Psychiatry **58**(2): 148-157.

Matheny, A. P., R. S. Wilson, et al. (1976). "RELATIONS BETWEEN TWINS SIMILARITY OF APPEARANCE AND BEHAVIORAL SIMILARITY - TESTING AN ASSUMPTION." Behavior Genetics **6**(3): 343-351.

Maxwell, M. E. (1992). Family Interview for Genetic Studies (FIGS): Manual for FIGS. Bethesda, MD, Clinical Neurogenetics Branch, Intramural Research Programme, National Institute of Mental Health.

McClellan, J. M., E. Susser, et al. (2007). "Schizophrenia: a common disease caused by multiple rare alleles." British Journal of Psychiatry **190**: 194-199.

McDonald, C., E. Bullmore, et al. (2005). "Regional volume deviations of brain structure in schizophrenia and psychotic bipolar disorder - Computational morphometry study." British Journal of Psychiatry **186**: 369-377.

McDonald, C., E. T. Bullmore, et al. (2004). "Association of Genetic Risks for Schizophrenia and Bipolar Disorder With Specific and Generic Brain Structural Endophenotypes." Arch Gen Psychiatry **61**: 974-984.

McDonald, C., E. T. Bullmore, et al. (2004). "Association of genetic risks for schizophrenia and bipolar disorder with specific and generic brain structural endophenotypes." Archives of General Psychiatry **61**(10): 974-984.

McDonald, C., A. Grech, et al. (2002). "Brain volumes in familial and non-familial schizophrenic probands and their unaffected relatives." American Journal of Medical Genetics **114**(6): 616-625.

McDonald, C., N. Marshall, et al. (2006). "Regional Brain Morphometry in Patients With Schizophrenia or Bipolar Disorder and Their Unaffected Relatives." American Journal of Psychiatry **163**(3): 478-487.

McDonald, C., N. Marshall, et al. (2006). "Regional brain morphometry in patients with schizophrenia or bipolar disorder and their unaffected relatives." American Journal of Psychiatry **163**(3): 478-487.

McDonald, P., M. Lewis, et al. (2003). "Appraisal of genetic and epigenetic congruity of a monozygotic twin pair discordant for schizophrenia." J Med Genet **40**(2): E16.

McEwen, B. S. (2008). "Understanding the potency of stressful early life experiences on brain and body function." Metabolism Clinical and Experimental **57**(Suppl 2): S11-S15.

McGrath, J. J. (2006). "Variations in the incidence of schizophrenia: Data versus dogma." Schizophrenia Bulletin **32**(1): 195-197.

McGrath, J. J., S. Saha, et al. (2004). "A systematic review of the incidence of schizophrenia: The distribution of rate items and the influence of methodology, urbanicity, sex and migrant status." Schizophrenia Research **67**(1): 65-66.

McGuffin, P., P. Asherson, et al. (1994). "The strength of the genetic effect - Is there room for an environmental influence in the etiology of schizophrenia." British Journal of Psychiatry **164**: 593-599.

McGuffin, P., A. Farmer, et al. (1987). "Is there really a split in schizophrenia - the genetic evidence." British Journal of Psychiatry **150**: 581-592.

McGuffin, P., A. Farmer, et al. (1991). "A Polydiagnostic application of operational criteria in studies of psychotic illness - Development and reliability of the OPCRIT system." Archives of General Psychiatry **48**(8): 764-770.

McGuffin, P., A. E. Farmer, et al. (1984). "Twin concordance for operationally defined schizophrenia - confirmation of familality and heritability." Archives of General Psychiatry **41**(6): 541-545.

McNeil, T. F., E. Cantor-Graae, et al. (2000). "Relationship of obstetric complications and differences in size of brain structures in monozygotic twin pairs discordant for schizophrenia." American Journal of Psychiatry **157**(2): 203-212.
McNeil, T. F., E. Cantorgraae, et al. (1994). "OBSTETRIC COMPLICATIONS AS ANTECEDENTS OF SCHIZOPHRENIA - EMPIRICAL EFFECTS OF USING DIFFERENT OBSTETRIC COMPLICATION SCALES." Journal of Psychiatric Research **28**(6): 519-530.
McNeil, T. F., E. Cantorgraae, et al. (1994). "OBSTETRIC COMPLICATIONS IN HISTORIES OF MONOZYGOTIC TWINS DISCORDANT AND CONCORDANT FOR SCHIZOPHRENIA." Acta Psychiatrica Scandinavica **89**(3): 196-204.
Mechelli, A., D. P. Prata, et al. (2008). "The effects of neuregulin1 on brain function in controls and patients with schizophrenia and bipolar disorder." Neuroimage **42**(2): 817-826.
Mechri, A., M.-C. Bourdel, et al. (2009). "Neurological soft signs in patients with schizophrenia and their unaffected siblings: frequency and correlates in two ethnic and socioeconomic distinct populations." Eur Arch Psychiatry Clin Neurosci **259**(4): 218-226.
Mechri, A., L. Gassab, et al. (2010). "Neurological soft signs and schizotypal dimensions in unaffected siblings of patients with schizophrenia." Psychiatry Research **175**(1-2): 22-26.
Meyer-Lindenberg, A., J. B. Poline, et al. (2001). "Evidence for abnormal cortical functional connectivity during working memory in schizophrenia." American Journal of Psychiatry **158**(11): 1809-1817.
Moncrieff, J. and J. Leo "A systematic review of the effects of antipsychotic drugs on brain volume." Psychological Medicine **40**(9): 1409-1422.
Moorhead, T. W. J., V. E. Gountouna, et al. (2009). "Prospective multi-centre Voxel Based Morphometry study employing scanner specific segmentations: Procedure development using CaliBrain structural MRI data." BMC Medical Imaging: 8 (20 pp.).
Morel, B. A. (1860). Traite des Maladies Mentales. Paris, Masson.
Morgan, C. and H. Fisher (2007). "Environment and schizophrenia: Environmental factors in schizophrenia: Childhood trauma - A critical review." Schizophrenia Bulletin **33**(1): 3-10.
Morris Yates, A., G. Andrews, et al. (1990). "TWINS - A TEST OF THE EQUAL ENVIRONMENTS ASSUMPTION." Acta Psychiatrica Scandinavica **81**(4): 322-326.
Mosher, L. R., W. Pollin, et al. (1970). "Family Studies of Mz Twins Discordant for Schizophrenia - Neurological Findings." Acta Geneticae Medicae Et Gemellologiae **19**(1-2): 305-&.
Mosher, L. R., W. Pollin, et al. (1971). "FAMILIES WITH IDENTICAL TWINS DISCORDANT FOR SCHIZOPHRENIA - SOME RELATIONSHIPS BETWEEN IDENTIFICATION, THINKING STYLES, PSYCHOPATHOLOGY AND DOMINANCE-SUBMISSIVENESS." British Journal of Psychiatry **118**(542): 29-42.
Mosher, L. R., W. Pollin, et al. (1971). "IDENTICAL TWINS DISCORDANT FOR SCHIZOPHRENIA - NEUROLOGIC FINDINGS." Archives of General Psychiatry **24**(5): 422-&.

Munoz Maniega, S., G. K. S. Lymer, et al. (2008). "A diffusion tensor MRI study of white matter integrity in subjects at high genetic risk of schizophrenia." Schizophr Res **106**(2-3): 132-139.
Murray, R. M., E. O'Callaghan, et al. (1992). "A Neurodevelopmental Approach to the Classification of Schizophrenia." Schizophrenia Bulletin **18**(2): 319-332.
Myin-Germeys, I., L. Krabbendam, et al. (2003). "Do life events have their effect on psychosis by influencing the emotional reactivity to daily life events?" Psychological Medicine **33**: 327-333.
Narr, K. L., T. D. Cannon, et al. (2002). "Genetic contributions to altered callosal morphology in schizophrenia." Journal of Neuroscience **22**(9): 3720-3729.
Narr, K. L., T. G. M. van Erp, et al. (2002). "A twin study of genetic contributions to hippocampal morphology in schizophrenia." Neurobiology of Disease **11**(1): 83-95.
Nathan, M. and R. Guttman (1984). "SIMILARITIES IN TEST-SCORES AND PROFILES OF KIBBUTZ TWINS AND SINGLETONS." Acta Geneticae Medicae Et Gemellologiae **33**(2): 213-218.
Navari, S. and P. Dazzan (2009). "Do antipsychotic drugs affect brain structure? A systematic and critical review of MRI findings." Psychological Medicine **39**(11): 1763-1777.
Nayani, T. H. and A. S. David (1996). "The auditory hallucination: A phenomenological survey." Psychological Medicine **26**(1): 177-189.
Nelson, B., A. R. Yung, et al. (2008). "The phenomenological critique and self-disturbance: Implications for ultra-high risk ("Prodrome") research." Schizophrenia Bulletin **34**(2): 381-392.
Nguyen, G. H., J. Bouchard, et al. (2003). "DNA stability and schizophrenia in twins." American Journal of Medical Genetics Part B-Neuropsychiatric Genetics **120B**(1): 1-10.
Niethammer, R., M. Weisbrod, et al. (2000). "Genetic influence on laterality in schizophrenia? A twin study of neurological soft signs." American Journal of Psychiatry **157**(2): 272-274.
Nieuwint, A., R. Van Zalen-Sprock, et al. (1999). "'Identical' twins with discordant karyotypes." Prenatal Diagnosis **19**(1): 72-76.
Niznikiewicz, M. A., M. Kubicki, et al. (2003). "Recent structural and functional imaging findings in schizophrenia." Current Opinion in Psychiatry **16**(2): 123-147.
Noga, J. T., A. J. Bartley, et al. (1996). "Cortical gyral anatomy and gross brain dimensions in monozygotic twins discordant for schizophrenia." Schizophrenia Research **22**(1): 27-40.
Nordgaard, J., S. M. Arnfred, et al. (2008). "The diagnostic status of first-rank symptoms." Schizophrenia Bulletin **34**(1): 137-154.
O'Daly, O. G., O. Guillin, et al. (2005). "Schizophrenia and substance abuse co-morbidity: a role for dopamine sensitisation?" Journal of Dual Diagnosis **1**(2): 11-40.
O'Donovan, M. C., N. Craddock, et al. (2008). "Identification of loci associated with schizophrenia by genome-wide association and follow-up." Nature Genetics **40**(9): 1053-1055.
Obladen, M. (2010). "Unequal but monozygous: a history of twin-twin transfusion syndrome." Journal of Perinatal Medicine **38**(2): 121-128.
Ohara, K., H. D. Xu, et al. (1998). "Cerebral ventricle-brain ratio in monozygotic twins discordant and concordant for schizophrenia." Progress in Neuro-Psychopharmacology & Biological Psychiatry **22**(6): 1043-1050.

Olin, S. S. and S. A. Mednick (1996). "Risk facotrs of psychosis: identifying populations premorbidly." Schizophrenia Bulletin **22**(2): 223-240.
Onstad, S., I. Skre, et al. (1991). "Subtypes of schizophrenia - evidence from a twin family study." Acta Psychiatrica Scandinavica **84**(2): 203-206.
Onstad, S., I. Skre, et al. (1992). "Birth-weight and obstetric complications in schizophrenic twins." Acta Psychiatrica Scandinavica **85**(1): 70-73.
Orstavik, R. E., N. Tommerup, et al. (1995). "NONRANDOM X-CHROMOSOME INACTIVATION IN AN AFFECTED TWIN IN A MONOZYGOTIC TWIN PAIR DISCORDANT FOR WIEDEMANN-BECKWITH SYNDROME." American Journal of Medical Genetics **56**(2): 210-214.
Owens, S. F., F. Rijsdijk, et al. (2010). "Genetic overlap between schizophrenia and selective components of executive function." Schizophrenia Research **In Press, Corrected Proof**.
Owens, S. F., F. Rijsdijk, et al. (In Press). "Genetic overlap between schizophrenia and selective components of executive function." Schizophr Res.
Oyebode, F. (2008). Sims Symptoms in the Mind, 4th Edition, An Introduction to Descriptive Psychopathology Saunders Elsevier
Pantelis, C., D. Velakoulis, et al. (2003). "Neuroanatomical abnormalities before and after onset of psychosis: a cross-sectional and longitudinal MRI comparison." Lancet **361**(9354): 281-288.
Pantelis, C., D. Velakoulis, et al. (2003). "Neuroanatomical abnormalities before and after onset of psychosis: a cross-sectional and longitudinal MRI comparison." The Lancet **361**(9354): 281-288.
Pardo, P. J., M. A. Knesevich, et al. (2000). "Genetic and state variables of neurocognitive dysfunction in schizophrenia: A twin study." Schizophrenia Bulletin **26**(2): 459-477.
Pearlson, G. D. (1997). "Superior temporal gyrus and planum temporale in schizophrenia: A selective review." Progress in Neuro-Psychopharmacology & Biological Psychiatry **21**(8): 1203-1229.
Pedersen, C. B. and P. B. Mortensen (2001). "Evidence of a dose-response relationship between urbanicity during upbringing and schizophrenia risk." Archives of General Psychiatry **58**(11): 1039-1046.
Pennington, B. F., P. A. Filipek, et al. (2000). "A twin MRI study of size variations in the human brain." Journal of Cognitive Neuroscience **12**(1): 223-232.
Petronis, A. and J. L. Kennedy (1995). "Unstable genes - unstable mind." American Journal of Psychiatry **152**(2): 164-172.
Petronis, A., A. D. Paterson, et al. (1999). "Schizophrenia: An epigenetic puzzle?" Schizophrenia Bulletin **25**(4): 639-655.
Petronis, A., J. B. Vincent, et al. (1998). "Search for unstable DNA in schizophrenia." Schizophrenia Research **29**(1-2): 135-135.
Phelps, E. A., F. Hyder, et al. (1997). "Fmri of the prefrontal cortex during overt verbal fluency." Neuroreport **8**: 561-565.
Phillips, D. I. W. (1993). "TWIN STUDIES IN MEDICAL-RESEARCH." Lancet **342**(8862): 52-52.
Phillips, L. J., A. R. Yung, et al. (2002). "Prediction and prevention of transition to psychosis in young people at incipient risk for schizophrenia." American Journal of Medical Genetics **114**(8): 929-937.
Picchioni, M. M. and R. M. Murray (2007). "Schizophrenia." British Medical Journal **335**(7610): 91-95.

Picchioni, M. M., T. Toulopoulou, et al. (2006). "Neurological Abnormalities in Schizophrenic Twins." Biological Psychiatry **59**(4): 341-348.
Picchioni, M. M., M. Walshe, et al. (2010). "Genetic modelling of childhood social development and personality in twins and siblings with schizophrenia." Psychological Medicine **40**(8): 1305-1316.
Pihlajamaki, M., H. Tanila, et al. (2000). "Verbal fluency activates the left medial temporal lobe: A functional magnetic resosnce imaging study." Annals of Neurology **47**: 470-476.
Pinborg, A., A. Loft, et al. (2004). "Neurological sequelae in twins born after assisted conception: controlled national cohort study." BMJ **329**: 311-315.
Plomin, R. and C. S. Bergeman (1991). "Nature and nurture - authors response." Behavioral and Brain Sciences **14**(3): 414-428.
Plomin, R. and C. S. Bergeman (1991). "The Nature of Nurture - Genetic Influence on Environmental Measures." Behavioral and Brain Sciences **14**(3): 373-385.
Plomin, R. and C. S. Bergeman (1991). "The nature of nurture - genetics of environmental measures." Behavioral and Brain Sciences **14**(3): 373-385.
Plomin, R., J. C. DeFries, et al., Eds. (1997). Behavioural Genetics. New York, W.H. Freeman and Company.
Plomin, R., L. Willerman, et al. (1976). "RESEMBLANCE IN APPEARANCE AND EQUAL ENVIRONMENTS ASSUMPTION IN TWIN STUDIES OF PERSONALITY-TRAITS." Behavior Genetics **6**(1): 43-52.
Pollin, W., J. R. Stabenau, et al. (1966). "LIFE HISTORY DIFFERENCES IN IDENTICAL TWINS DISCORDANT FOR SCHIZOPHRENIA." American Journal of Orthopsychiatry **36**(3): 492-510.
Pollin, W., J. R. Stabenau, et al. (1965). "FAMILY STUDIES WITH IDENTICAL-TWINS DISCORDANT FOR SCHIZOPHRENIA." Psychiatry **28**(1): 60-78.
Poltorak, M., R. Wright, et al. (1997). "Monozygotic twins discordant for schizophrenia are discordant for N-CAM and L1 in CSF." Brain Research **751**(1): 152-154.
Polymeropoulos, M. H., H. Xiao, et al. (1993). "SEARCH FOR A GENETIC EVENT IN MONOZYGOTIC TWINS DISCORDANT FOR SCHIZOPHRENIA." Psychiatry Research **48**(1): 27-36.
Porteous, D. J., P. Thomson, et al. (2006). "The Genetics and Biology of Disc1--An Emerging Role in Psychosis and Cognition." Biological Psychiatry **60**(2): 123-131.
Posthuma, D., E. J. de Geus, et al. (2000). "Multivariate genetic analysis of brain structure in an extended twin design." Behaviour Genetics **30**(4): 311-319.
Poulton, R., A. Caspi, et al. (2000). "Children's self-reported psychotic symptoms and adult schizophreniform disorder - A 15-year longitudinal study." Archives of General Psychiatry **57**(11): 1053-1058.
Prasad, K. M. and M. S. Keshavan (2008). "Structural cerebral variations as useful endophenotypes in schizophrenia: Do they help construct "Extended Endophenotypes"?" Schizophrenia Bulletin **34**(4): 774-790.
Prasad, K. M., R. Sanders, et al. (2009). "Neurological abnormalities among offspring of persons with schizophrenia: Relation to premorbid psychopathology." Schizophrenia Research **108**(1-3): 163-169.
Prata, D. P., A. Mechelli, et al. (2009). "Opposite Effects of Catechol-O-Methyltransferase Val158Met on Cortical Function in Healthy Subjects and Patients with Schizophrenia." Biological Psychiatry **65**(6): 473-480.
Prata, D. P., A. Mechelli, et al. (2008). "Effects of disrupted-in-schizophrenia-1 on pre-frontal cortical function." Molecular Psychiatry **13**: 915-917.

Prata, D. P., A. Mechelli, et al. (2009). "Epistasis between the DAT 3 ' UTR VNTR and the COMT Val158Met SNP on cortical function in healthy subjects and patients with schizophrenia." Proceedings of the National Academy of Sciences of the United States of America **106**(32): 13600-13605.
Prata, D. P., A. Mechelli, et al. (2009). "Altered Effect of Dopamine Transporter 3 ' UTR VNTR Genotype on Prefrontal and Striatal Function in Schizophrenia." Archives of General Psychiatry **66**(11): 1162-1172.
Preston, G. A. and D. R. Weinberger (2005). "Intermediate phenotypes in schizophrenia: a selective review." Dialogues Clin Neurosci **7**(2): 165-179.
Ragins, N., J. Schachter, et al. (1975). "Infants and children at risk for schizophrenia: environmental and developmental observations." American Journal of Child Psychiatry **14**: 150-177.
Rajarethinam, R., S. Sahni, et al. (2004). "Reduced superior temporal gyrus volume in young offspring of patients with schizophrenia." American Journal of Psychiatry **161**(6): 1121-1124.
Raschka, L. B. (2000). "Paternal age and schizophrenia in dizygotic twins." British Journal of Psychiatry **176**: 400-401.
Rebollo, I. and D. I. Boomsma (2006). "Genetic analysis of anger: Genetic dominance or competitive sibling interaction." Behavior Genetics **36**(2): 216-228.
Reichenberg, A. and P. D. Harvey (2007). "Neuropsychological impairments in schizophrenia: Integration of performance-based and brain imaging findings." Psychological Bulletin **133**: 833-858.
Reichenberg, A., J. Rabinowitz, et al. (2000). "Premorbid functioning in a national population of male twins discordant for psychoses." American Journal of Psychiatry **157**(9): 1514-1516.
Reichenberg, A., M. Weiser, et al. (1999). "Premorbid personality and neuropsychological assessment of male twins discordant for schizophrenia." Schizophrenia Research **36**(1-3): 151-151.
Rettew, D. C., I. Rebollo-Mesa, et al. (2008). "Non-additive and additive genetic effects on extraversion in 3314 Dutch adolescent twins and their parents." Behavior Genetics **38**(3): 223-233.
Reveley, A. M., M. A. Reveley, et al. (1982). "Cerebral ventricle size in twins discordant for schizophrenia." Lancet **1**: 540-541.
Reveley, A. M., M. A. Reveley, et al. (1982). "CEREBRAL VENTRICULAR SIZE IN TWINS DISCORDANT FOR SCHIZOPHRENIA." Lancet **1**(8271): 540-541.
Reveley, A. M., M. A. Reveley, et al. (1984). "CEREBRAL VENTRICULAR ENLARGEMENT IN NON-GENETIC SCHIZOPHRENIA - A CONTROLLED TWIN STUDY." British Journal of Psychiatry **144**(JAN): 89-93.
Rey, M. J., P. Schulz, et al. (1989). "GUIDELINES FOR THE DOSAGE OF NEUROLEPTICS .1. CHLORPROMAZINE EQUIVALENTS OF ORALLY-ADMINISTERED NEUROLEPTICS." International Clinical Psychopharmacology **4**(2): 95-104.
Rijsdijk, F. V., N. E. M. van Haren, et al. (2005). "Brain MRI Abnormalities in Schizophrenia: Same Genes or Same Environment?" Psychological Medicine **35**(10): 1399-1409.
Rijsdijk, F. V., N. E. M. Van Haren, et al. (2005). "Brain MRI abnormalities in schizophrenia: same genes or same environment?" Psychological Medicine **35**(10): 1399-1409.
Ritsner, M. S. and I. I. Gottesman (2009). "Where Do We Stand in the Quest for Neuropsychiatric Biomarkers and Endophenotypes and What Next?" Handbook

of Neuropsychiatric Biomarkers, Endophenotypes and Genes, Vol 1:NEUROPSYCHOLOGICAL ENDOPHENOTYPES AND BIOMARKERS: 3-21.
Rogers, J. G., L. Voullaire, et al. (1982). "MONOZYGOTIC TWINS DISCORDANT FOR TRISOMY 21." American Journal of Medical Genetics **11**(2): 143-146.
Rogers, W. H. (1993). "Regression standard errors in clustered samples." Reprinted in Stata Technical Bulleting Reprints **3**: 88-94.
Rosa, A., L. Fananas, et al. (2000). "Congenital dermatoglyphic malformations and psychosis: A twin study." American Journal of Psychiatry **157**(9): 1511-1513.
Rosenthal, D. (1959). "Some factors associated with concordance and discordance with respect to schizophrenia in monozygotoc twins." Journal of Nervous and Mental Disease **129**(1): 1-10.
Rosenthal, D. (1960). "Confusion of Identity and the Frequency of Schizophrenia in Twins." Archives of General Psychiatry **3**(3): 297-304.
Rosenthal, D. and S. S. Kety, Eds. (1968). The transmission of Schizophrenia. Oxford, Pergamon Press.
Rossi, A., S. Decataldo, et al. (1990). "Neurological Soft Signs in Schizophrenia." British Journal of Psychiatry **157**: 735-739.
Sadrzadeh, S., S. A. Treloar, et al. (2001). "Potential bias regarding birth weight in historical and contemporary twin data bases." Twin Res **4**(5): 332-336.
Saha, S., D. Chant, et al. (2005). "A systematic review of the prevalence of schizophrenia." Plos Medicine **2**(5): 413-433.
Samuels, L. (1978). "Sex- differences in concordance rates for schizophrenia - finding or artifact." Schizophrenia Bulletin **4**(1): 14-15.
Samuels, L. (1979). "Sex-differences in schizophrenia - reply." Schizophrenia Bulletin **5**(1): 8-10.
Sartorius, N., R. Shapiro, et al. (1974). "The International Pilot Study of Schizophrenia." Schizophrenia Bulletin **11**: 21-34.
Scarr, S. (1968). "Environmental bias in twin studies." Eugenics Quarterly **15**(1): 34-40.
Scarr, S. (1996). "How people make their own environments: Implications for parents and policy makers." Psychology Public Policy and Law **2**(2): 204-228.
Scarr, S. and K. McCartney (1983). "How people make their environments - a theory of genotype environment effects." Child Development **54**(2): 424-435.
Scheepers, F. E., C. C. G. de Wied, et al. (2001). "Effect of clozapine on caudate nucleus volume in relation to symptoms of schizophrenia." American Journal of Psychiatry **158**(4): 644-646.
Schlosser, R., M. Hutchinson, et al. (1998). "Functional magnetic resonance imaging of human brain activity in a verbal fluency task." Journal of Neurology Neurosurgery and Psychiatry **64**: 492-498.
Schmahmann, J. D., J. Doyon, et al. (1999). "Three-dimensional MRI atlas of the human cerebellum in proportional stereotaxic space." Neuroimage **10**(3): 233-260.
Schnack, H. G., N. E. M. van Haren, et al. (2004). "Reliability of brain volumes from multicenter MRI acquisition: A calibration study." Human Brain Mapping **22**(4): 312-320.
Schubert, E. W., E. Cantor-Graae, et al. (2002). "Neurological deviation in offspring of women with psychosis: From infancy to adulthood." Schizophrenia Research **53**(3): 232-232.
Schubert, E. W. and T. F. McNeil (2004). "Prospective study of neurological abnormalities in offspring of women with psychosis: Birth to adulthood." American Journal of Psychiatry **161**(6): 1030-1037.

Schulze, K., C. McDonald, et al. (2003). "Hippocampal volume in familial and nonfamilial schizophrenic probands and their unaffected relatives." Biological Psychiatry **53**(7): 562-570.
Seeman, M. V. (2009). "The Changing Role of Mother of the Mentally Ill: From Schizophrenogenic Mother to Multigenerational Caregiver." Psychiatry-Interpersonal and Biological Processes **72**(3): 284-294.
Seidman, L. J., S. V. Faraone, et al. (2002). "Left hippocampal volume as a vulnerability indicator for schizophrenia - A magnetic resonance imaging morphometric study of nonpsychotic first-degree relatives." Archives of General Psychiatry **59**(9): 839-849.
Selten, J. P., J. D. Blom, et al. (2008). "Psychosis risk for parents and siblings of Dutch and Moroccan-Dutch patients with non-affective psychotic disorder." Schizophrenia Research **104**(1-3): 274-278.
Selten, J. P. and E. Cantor-Graae (2010). "The denial of a psychosis epidemic." Psychol Med **40**(5): 731-733.
Shapiro, D. I., S. Marcenco, et al. (2009). "The Premorbid Adjustment Scale as a measure of developmental compromise in patients with schizophrenia and their healthy siblings." Schizophrenia Research **In Press**.
Sharma, T., E. Lancaster, et al. (1998). "Brain changes in schizophrenia - Volumetric MRI study of families multiply affected with schizophrenia - The Maudsley Family Study 5." British Journal of Psychiatry **173**: 132-138.
Shen, S., A. J. Szameitat, et al. (2007). "VBM lesion detection depends on the normalization template: a study using simulated atrophy." Magnetic Resonance Imaging **25**(10): 1385-1396.
Shenton, M. E., C. C. Dickey, et al. (2001). "A review of MRI findings in schizophrenia." Schizophrenia Research **49**(1-2): 1-52.
Shibre, T., D. Kebede, et al. (2002). "Neurological soft signs (NSS) in 200 treatment-naive cases with schizophrenia: A community-based study in a rural setting." Nordic Journal of Psychiatry **56**(6): 425-431.
Shim, G., D. H. Kang, et al. (2008). "Social functioning deficits in young people at risk for schizophrenia." Australian and New Zealand Journal of Psychiatry **42**(8): 678-685.
Singh, S., B. Murphy, et al. (2002). "Epigenetic contributors to the discordance of monozygotic twins." Clinical Genetics **62**(2): 97-103.
Sirugo, G., J. Ashenbrenner, et al. (2004). "No evidence of association between the genetic predisposition for dizygotic twinning and schizophrenia in West Africa." Schizophrenia Research **70**(2-3): 343-344.
Sklar, P. (2002). "Linkage analysis in psychiatric disorders: the emerging picture." Annual Review of Genomics and Human Genetics **25**: 1-50.
Slaghekke, F., W. J. Kist, et al. (2009). "TAPS and TOPS: two distinct forms of feto-fetal transfusion in monochorionic twins." Z Geburtshilfe Neonatol **213**(6): 248-254.
Slater, E. (1953). "Psychotic and neurotic illnesses in twins." Spec Rep Ser Med Res Counc (G B) **278**: 1-385.
Smieskova, R., P. Fusar-Poli, et al. (2009). "The Effects of Antipsychotics on the Brain: What Have We Learnt from Structural Imaging of Schizophrenia? - A Systematic Review." Current Pharmaceutical Design **15**(22): 2535-2549.
Sobel, D. (1961). "Children of schizophrenic patients: preliminary observations on early development." American Journal of Psychiatry **118**: 512-517.
Sommer, I. E. C., N. F. Ramsey, et al. (2001). "Language lateralization in schizophrenia, an fMRI study." Schizophrenia Research **52**(1-2): 57-67.

Sommer, I. E. C., N. F. Ramsey, et al. (2004). "Language activation in monozygotic twins discordant for schizophrneia." British Journal of Psychiatry **184**: 128-135.
Sommer, I. E. C., N. F. Ramsey, et al. (2003). "Language lateralization in female patients with schizophrenia: an fMRI study." Schizophrenia Research **60**(2-3): 183-190.
Sommer, I. E. C., N. F. Ramsey, et al. (2004). "Language activation in monozygotic twins discordant for schizophrenia." British Journal of Psychiatry **184**: 128-135.
Spaniel, F., T. Hajek, et al. (2003). "Differences in fMRI and MRS in a monozygotic twin pair discordant for schizophrenia (case report)." Acta Psychiatrica Scandinavica **107**(2): 155-157.
Spaniel, F., V. Herynek, et al. (2005). "Magnetic resonance relaxometry in monozygotic twins discordant and concordant for schizophrenia." European Psychiatry **20**(1): 41-44.
Spaniel, F., J. Tintera, et al. (2007). "Language lateralization in monozygotic twins discordant and concordant for schizophrenia. A functional MRI pilot study." European Psychiatry **22**(5): 319-322.
Spence, S. A., P. F. Liddle, et al. (2000). "Functional anatomy of verbal fluency in people with schizophrenia and those at genetic risk - Focal dysfunction and distributed disconnectivity reappraised." British Journal of Psychiatry **176**: 52-60.
Spitz, L. (2005). "Conjoined twins." Prenatal Diagnosis **25**(9): 814-819.
Spitzer, R. L., J. Endicott, et al. (1978). "Research diagnostic criteria: rationale and reliability." Arch Gen Psychiatry **35**(6): 773-782.
Staal, W. G., H. E. H. Pol, et al. (2000). "Structural brain abnormalities in patients with schizophrenia and their healthy siblings." American Journal of Psychiatry **157**(3): 416-421.
Stabenau, J. R. and W. Pollin (1967). "EARLY CHARACTERISTICS OF MONOZYGOTIC TWINS DISCORDANT FOR SCHIZOPHRENIA." Archives of General Psychiatry **17**(6): 723-&.
Stabenau, J. R., W. Pollin, et al. (1969). "Study of monozygotic twins discordant for schizophrenia - some biologic variables (lactate-pyruvic ratio - 3,4-dimethoxyphenlethylamine - S19 macroglobulin - antirabbit hemagglutin - protein bound iodine)."
Stassen, H. H., R. Coppola, et al. (1999). "EEG differences in monozygotic twins discordant and concordant for schizophrenia." Psychophysiology **36**(1): 109-117.
Stefansson, H., R. A. Ophoff, et al. (2009). "Common variants conferring risk of schizophrenia." Nature **460**(7256): 744-U799.
Stefansson, H., E. Sigurdsson, et al. (2002). "Neuregulin 1 and susceptibility to schizophrenia." American Journal of Human Genetics **71**(4): 877-892.
Stephan, K. E., T. Baldeweg, et al. (2006). "Synaptic plasticity and dysconnection in schizophrenia." Biological Psychiatry **59**(10): 929-939.
Stephan, K. E., K. J. Friston, et al. (2009). "Dysconnection in Schizophrenia: From Abnormal Synaptic Plasticity to Failures of Self-monitoring." Schizophrenia Bulletin **35**(3): 509-527.
Stern, A. J., A. A. Savostyanova, et al. (2008). "Impact of the Brain-Derived Neurotrophic Factor Val66Met Polymorphism on Levels of Hippocampal N-Acetyl-Aspartate Assessed by Magnetic Resonance Spectroscopic Imaging at 3 Tesla." Biological Psychiatry **64**(10): 856-862.
Straub, R. E. and D. R. Weinberger (2006). "Schizophrenia Genes - Famine to Feast." Biological Psychiatry **60**(2): 81-83.
Styner, M., J. A. Lieberman, et al. (2005). "Morphometric analysis of lateral ventricles in schizophrenia and healthy controls regarding genetic and disease-specific

factors." Proceedings of the National Academy of Sciences of the United States of America **102**(13): 4872-4877.
Suddath, R. L., G. W. Christison, et al. (1990). "ANATOMICAL ABNORMALITIES IN THE BRAINS OF MONOZYGOTIC TWINS DISCORDANT FOR SCHIZOPHRENIA." New England Journal of Medicine **322**(12): 789-794.
Suddath, R. L., G. W. Christison, et al. (1990). "Anatomical abnormalities in the brains of monozygotic twins discordant for schizophrenia." New England Journal of Medicine **322**: 789-794.
Suhr, J. A. and M. B. Spitnagel (2001). "Factor versus cluster models of schizotypal traits. I: A comparison of unselected and highyl schizotypal samples." Schizophrenia Research **52**: 231-239.
Sullivan, P. F., K. S. Kendler, et al. (2003). "Schizophrenia as a complex trait - Evidence from a meta- analysis of twin studies." Archives of General Psychiatry **60**(12): 1187-1192.
Sullivan, P. F., K. S. Kendler, et al. (2003). "Schizophrenia as a complex trait - Evidence from a meta-analysis of twin studies." Archives of General Psychiatry **60**(12): 1187-1192.
Suzuki, S. (2009). "Impact of Perinatal Outcome of the Presence of Fused or Separate Placentas in Dichorionic Twins." Fetal Diagnosis and Therapy **26**(3): 127-130.
Szasz, T. (1996). "'Audible thoughts' and 'speech defect' in schizophrenia - A note on reading and translating Bleuler." British Journal of Psychiatry **168**(5): 533-535.
Szasz, T. (2008). "Debunking antipsychiatry: Laing, law, and largactil." Current Psychology **27**(2): 79-101.
Szasz, T. S. (1976). "Schizophrenia: the sacred symbol of psychiatry." Seishin Shinkeigaku Zasshi **78**(4): 261-308.
Talairach, J., P. Tournoux, et al. (1988). "Anatomical Stereotaxic Studies of the Frontal-Lobe in the Management of the Epilepsies." Epilepsia **29**(2): 205-205.
Tandon, R., M. S. Keshavan, et al. (2008). "Schizophrenia, "Just the Facts" What we know in 2008. 2. Epidemiology and etiology." Schizophrenia Research **102**(1-3): 1-18.
Tarbox, S. I. and M. F. Pogue-Geile (2008). "Development of social functioning in preschizophrenia children and adoescents: a systematic review." Psychological Bulletin **34**(4): 561-583.
Taylor, D., C. Paton, et al. (2009). The Maudsley Prescribing Guidelines, 10th Edition. London, Martin Dunitz.
Tepest, R., L. Wang, et al. (2003). "Hippocampal deformities in the unaffected siblings of schizophrenia subjects." Biological Psychiatry **54**(11): 1234-1240.
Thompson, P., T. D. Cannon, et al. (2002). "Mapping genetic influences on human brain structure." Annals of Medicine **34**(7-8): 523-536.
Thompson, P. M., G. Bartzokis, et al. (2009). "Time-Lapse Mapping of Cortical Changes in Schizophrenia with Different Treatments." Cerebral Cortex **19**(5): 1107-1123.
Tienari, P. (1963). "PSYCHIATRIC ILLNESSES IN IDENTICAL-TWINS." Acta Psychiatrica Scandinavica **39**: 9-&.
Torgersen, S., S. Onstad, et al. (1993). "True schizotypal personality disorder - A study of co-twins and relatives of schizophrenic probands." American Journal of Psychiatry **150**(11): 1661-1667.
Torrey, E. F. (1990). "Offspring of twins with schizophrenia." Archives of General Psychiatry **47**(10): 976-977.

Torrey, E. F., I. I. Gottesman, et al. (1994). Schizophrenia and Manic Depressive Disorder: The Biological Roots of Mental Illness as Revealed by a Landmark Study of Identical Twins. New York, Basic Books.
Toulopoulou, T., T. E. Goldberg, et al. (2010). "Impaired intellect and memory: a missing link between genetic risk and schizophrenia?" Arch Gen Psychiatry **67**(9): 905-913.
Toulopoulou, T., M. Picchioni, et al. (2007). "Substantial genetic overlap between neurocognition and schizophrenia - Genetic modeling in twin samples." Archives of General Psychiatry **64**: 1348-1355.
Toulopoulou, T., M. Picchioni, et al. (2007). "Substantial genetic overlap between neurocognition and schizophrenia: Genetic modeling in twin samples." Archives of General Psychiatry **64**(12): 1348-1355
Toulopoulou, T., M. M. Picchioni, et al. (2007). "Substantial Genetic Overlap Between Neurocognition and Schizophrenia." Arch Gen Psychiatry **64**(12): 1348-1355.
Tramo, M. J., W. C. Loftus, et al. (1995). "SURFACE-AREA OF HUMAN CEREBRAL-CORTEX AND ITS GROSS MORPHOLOGICAL SUBDIVISIONS - IN-VIVO MEASUREMENTS IN MONOZYGOTIC TWINS SUGGEST DIFFERENTIAL HEMISPHERE EFFECTS OF GENETIC-FACTORS." Journal of Cognitive Neuroscience **7**(2): 292-302.
Tsujita, T., N. Niikawa, et al. (1998). "Genomic discordance between monozygotic twins discordant for schizophrenia." American Journal of Psychiatry **155**(3): 422-424.
Uchida, I. A., D. J. Desa, et al. (1983). "45,X/46,XX MOSAICISM IN DISCORDANT MONOZYGOTIC TWINS." Pediatrics **71**(3): 413-417.
Valsky, D. V., E. Eixarch, et al. (2010). "Selective intrauterine growth restriction in monochorionic diamniotic twin pregnancies." Prenatal Diagnosis **30**(8): 719-726.
van Beilen, M., M. Pijnenborg, et al. (2004). "What is measured by verbal fluency tests in schizophrenia?" Schizophrenia Research **69**(2-3): 267-276.
van Erp, T. G. M., P. A. Saleh, et al. (2004). "Hippocampal volumes in schizophrenic twins." Archives of General Psychiatry **61**(4): 346-353.
van Erp, T. G. M., P. A. Saleh, et al. (2001). "Hippocampus volumes in schizophrenic patients, their siblings, and controls: Contributions of genes and fetal hypoxia." Neuroimage **13**(6): S1110-S1110.
van Haren, N. E. M., M. M. Picchioni, et al. (2004). "A Controlled Study of Brain Structure in Monozygotic Twins Concordant and Discordant for Schizophrenia." Biological Psychiatry **56**(6): 454-461.
van Haren, N. E. M., M. M. Picchioni, et al. (2004). "A controlled study of brain structure in monozygotic twins concordant and discordant for schizophrenia." Biological Psychiatry **56**(6): 454-461.
van Oel, C. J., W. F. C. Baare, et al. (2001). "Differentiating between low and high susceptibility to schizophrenia in twins: the significance of dermatoglyphic indices in relation to other determinants of brain development." Schizophrenia Research **52**(3): 181-193.
Van Oel, C. J., W. F. C. Baare, et al. (1999). "Genetic liability versus intrauterine environment in twins discordant for schizophrenia." Schizophrenia Research **36**(1-3): 213-214.
Van Oel, C. J. and R. S. Kahn (1997). "Birth order and low birth weight as predictors for schizophrenia in discordant twins." American Journal of Medical Genetics **74**(6): 566-566.
van Os, J., L. Fananas, et al. (1997). "Dermatoglyphic abnormalities in psychosis: A twin study." Biological Psychiatry **41**(5): 624-626.

van Os, J. and S. Kapur (2009). "Schizophrenia." Lancet **374**(9690): 635-645.
van Os, J., G. Kenis, et al. (2010). "The environment and schizophrenia." Nature **468**(7321): 203-212.
van Os, J., H. Verdoux, et al. (1999). "Self-reported psychosis-like symptoms and the continuum of psychosis." Social Psychiatry and Psychiatric Epidemiology **34**(9): 459-463.
Vander Putten, D. M., E. Fuller Torry, et al. (1996). "Plasma protein variations in monozygotic twins discordant for schizophrenia." Biological Psychiatry **40**: 437-442.
Veiling, W., E. S. Susser, et al. (2008). "Ethnic density of neighbourhoods and incidence of psychotic disorders among immigrants." American Journal of Psychiatry **165**: 66-73.
Veling, W., J. P. Selten, et al. (2007). "Discrimination and the incidence of psychotic disorders among ethnic minorities in The Netherlands." International Journal of Epidemiology **36**: 761-768.
Veling, W., E. Susser, et al. (2008). "Ethnic density of Neighborhoods and incidence of psychotic disorders among immigrants." American Journal of Psychiatry **165**(1): 66-73.
Vincent, J. B., G. Kalsi, et al. (1998). "No evidence of expansion of CAG or GAA repeats in schizophrenia families and monozygotic twins." Human Genetics **103**(1): 41-47.
Vinogradov, S., J. Kirkland, et al. (2003). "Both processing speed and semantic memory organization predict verbal fluency in schizophrenia." Schizophrenia Research **59**(2-3): 269-275.
Vojvoda, D., K. Grimmell, et al. (1996). "Monozygotic twins concordant for response to clozapine." Lancet **347**(8993): 61.
Vollema, M. G. and R. J. van den Bosch (1995). "The multidimensionality of schizotypy." Schizophrenia Bulletin **21**: 19-30.
Vourdas, A., R. Pipe, et al. (2003). "Increased developmental deviance and premorbid dysfunction in early onset schizophrenia." Schizophrenia Research **62**(1-2): 13-22.
Waddington, J. L., A. P. Corvin, et al. (2007). "Functional genomic and schizophrenia: endophenotypes and mutant models." Psychiatric Clinics of North America **30**(3): 365-399.
Walker, E. and D. Diforio (1997). "Schizophrenia: a neural diathesis-stress model." Psychological Review **104**(4): 667-685.
Walker, E., V. Mittal, et al. (2008). "Stress and the hypothalamic pituitary adrenal axis in the developmental course of schizophrenia." Annual Review of Clinical Psychology **4**: 189-216.
Walshe, M., M. Taylor, et al. (2007). "Familial liability to schizophrenia and premorbid adjustment." British Journal of Psychiatry **191**: 260-261.
Walters, J. T. R. and N. Craddock (2007). "Endophenotypes in psychiatric genetics." Molecular Psychiatry **12**: 886-890.
Warburton, E., R. J. S. Wise, et al. (1996). "Noun and verb retrieval by normal subjects studies with PET." Brain **119**: 159-179.
Weinberger, D. R. (1987). "Implications of normal brain development for the pathogenesis of schizophrenia." Archives of General Psychiatry **44**: 660-669.
Weinberger, D. R., K. F. Berman, et al. (1992). "EVIDENCE OF DYSFUNCTION OF A PREFRONTAL-LIMBIC NETWORK IN SCHIZOPHRENIA - A MAGNETIC-RESONANCE-IMAGING AND REGIONAL CEREBRAL BLOOD-FLOW

STUDY OF DISCORDANT MONOZYGOTIC TWINS." American Journal of Psychiatry **149**(7): 890-897.
Weisbrod, M., H. Hill, et al. (1999). "Genetic influence on auditory information processing in schizophrenia: P300 in monozygotic twins." Biological Psychiatry **46**(5): 721-725.
Weisbrod, M., H. Hill, et al. (2004). "Nongenetic Pathologic developments of brain-wave patterns in monozygotic twins discordant and concordant for schizophrenia." American Journal of Medical Genetics Part B-Neuropsychiatric Genetics **125B**(1): 1-9.
Weiss, E. M., A. Hofer, et al. (2004). "Brain activation patterns during a verbal fluency test - a functional MRI study in healthy volunteers and patients with schizophrenia." Schizophrenia Research **70**(2-3): 287-291.
Weiss, E. M., A. Hofer, et al. (2006). "Language lateralization in unmedicated patients during an acute episode of schizophrenia: a functional MRI study." Psychiatry Research **146**: 185-190.
Whalley, H. C., E. Simonotto, et al. (2004). "FMRI correlates of state and trait effects in subjects at genetically enhanced risk of schizophrenia." Brain **127**: 478-490.
Whalley, H. C., M. C. Whyte, et al. (2005). "Neural correlates of enhanced genetic risk for schizophrenia." Neuroscientist **11**(3): 238-249.
White, T., N. C. Andreasen, et al. (2002). "Brain volumes and surface morphology in monozygotic twins." Cerebral Cortex **12**(5): 486-493.
White, T., D. O'Leary, et al. (2001). "Anatomic and functional variability: The effects of filter size in group fMRI data analysis." NeuroImage **13**(4): 577-588.
Whyte, M. C., H. C. Whalley, et al. (2006). "Event-related fmri of word classification and successful word recognition in subjects at genetically enhanced risk of schizophrenia." Psychological Medicine **36**: 1427-1439.
Williams, N. M., A. Preece, et al. (2004). "Identification in 2 independent samples of a novel schizophrenia risk haplotype of the dystrobrevin binding protein gene (DTNBP1)." Archives of General Psychiatry **61**(4): 336-344.
Williams, R. L. (2000). "A note on robust variance estimation for cluster-correlated data." Biometrics **56**(2): 645-646.
Wojcik, J. D., A. J. Gelenberg, et al. (1980). "Prevalence of tardive dyskinesia in an outpatient population." Compr Psychiatry **21**(5): 370-380.
Wolff, A. L. and G. A. O'Driscoll (1999). "Motor deficits and schizophrenia: the evidence from neuroleptic-naive patients and populations at risk." Journal of Psychiatry & Neuroscience **24**(4): 304-314.
Wood, S. J., D. Velakoulis, et al. (2001). "A longitudinal study of hippocampal volume in first episode psychosis and chronic schizophrenia." Schizophrenia Research **52**(1-2): 37-46.
Woods, B. T. (1998). "Is schizophrenia a progressive neurodevelopmental disorder? Toward a unitary pathogenetic mechanism." American Journal of Psychiatry **155**(12): 1661-1670.
Woods, S. W. (2003). "Chlorpromazine equivalent doses for the newer atypical antipsychotics." Journal of Clinical Psychiatry **64**(6): 663-667.
Woodward, T. S., C. C. Ruff, et al. (2003). "Methodological considerations regarding the association of Stroop and verbal fluency performance with the symptoms of schizophrenia." Schizophrenia Research **61**(2-3): 207-214.
Wright, I. C., S. Rabe-Hesketh, et al. (2000). "Meta-analysis of regional brain volumes in schizophrenia." American Journal of Psychiatry **157**(1): 16-25.

Wright, I. C., P. Sham, et al. (2002). "Genetic contributions to regional variability in human brain structure: methods and preliminary analysis." NeuroImage **17**: 256-271.

Yazici, A. H., B. Demir, et al. (2002). "Neurological soft signs in schizophrenic patients and their nonpsychotic siblings." Schizophrenia Research **58**(2-3): 241-246.

Yung, A. R., L. J. Phillips, et al. (1998). "Can we predict the onset of first-episode psychosis in a high-risk group?" International Clinical Psychopharmacology **13**: S23-S30.

Yung, A. R., L. J. Phillips, et al. (2004). "Risk factors for psychosis in an ultra high-risk group: psychopathology and clinical features." Schizophrenia Research **67**(2-3): 131-142.

YurgelunTodd, D. A., C. M. Waternaux, et al. (1996). "Functional magnetic resonance imaging of schizophrenic patients and comparison subjects during word production." American Journal of Psychiatry **153**(2): 200-205.

Zandi, T., J. M. Havenaar, et al. (2010). "First contact incidence of psychotic disorders among native Dutch and Moroccan immigrants in the Netherlands: Influence of diagnostic bias." Schizophrenia Research **119**(1-3): 27-33.

Zumarraga, M., I. Andia, et al. (2004). "Expression in normals and in subjects with schizophrenia of a novel gene fragment originally isolated from monozygotic twins discordant for schizophrenia." Genetics and Molecular Biology **27**(1): 17-21.

Zwijnenburg, P. J. G., H. Meijers-Heijboer, et al. (2010). "Identical But Not the Same: The Value of Discordant Monozygotic Twins in Genetic Research." American Journal of Medical Genetics Part B-Neuropsychiatric Genetics **153B**(6): 1134-1149.